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(54) Title: NOVEL SURFACE PROTEIN OF NEISSERIA MENINGITIDIS

#### (57) Abstract

The invention provides a novel surface polypeptide from Neisseria meningitidis as well as nucleic acid and nucleic acid sequence homologues encoding this protein. Pharmaceutical compositions containing the polypeptide and nucleic acids of the invention are also disclosed as well as methods useful in the treatment, prevention and diagnosis of N. meningitidis infection.

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#### TITLE

#### "NOVEL SURFACE ANTIGEN"

### FIELD OF THE INVENTION

The present invention relates to novel polypeptides as for example obtainable from Neisseria meningitidis, to nucleotide sequences encoding such polypeptides, to the use of these in diagnostics, in therapeutic and prophylactic vaccines and in the design and/or screening of medicaments.

## BACKGROUND OF THE INVENTION

Neisseria meningitidis is a Gram-negative bacterium and the causative agent of meningococcal meningitis and septicemia. Its only known host is the human, and it may be carried asymptomatically by approximately 10% of the population (Caugant, D. et al, 1994, Journal of Clinical Microbiology, 32:323-30).

N. meningitidis may express a polysaccharide allows classification this capsule, bacteria according to the nature of the capsule expressed. There are at least thirteen serogroups of N. meningitidis: A,B,C,29-E,H,I,K,L,W135,X,Y and Z, of cause 90% В, and C serogroups Α, which meningococcal disease (Poolman, J.T. et Infectious Agents and Disease, 4:13-28). Vaccines directed against serogroups A and C are available, but the serogroup B capsular polysaccharide is poorly immunogenic and does not induce protection in humans.

Other membrane and extracellular components are therefore being examined for their suitability for

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inclusion in vaccines. Examples include the outer membrane proteins of classes 1, 2 and 3 (porins), and classes 4 (Rmp) and 5 (Opacity proteins). However, to date, none of these candidates is able to induce complete protection, particularly in children (Romero, J.D., 1994, Clinical Microbiology Review, 7:559-575; Poolman, J.T. et al, 1995, supra).

create an effective vaccine, To necessary to identify components of N. meningitidis which are present in a majority of strains, and which are capable of inducing a protective immune response (bactericidal antibodies). In this regard, reference al. (International Brodeur et be made may Publication WO 96/29412) who disclose a 22 kDa surface protein which is highly conserved across 99% of all known strains of N. meningitidis. Injection of purified recombinant 22 kDa surface protein protected 80% of immunized mice against development of a lethal infection by N. meningitidis. Notwithstanding the discovery of this protein, there is still a need to isolate more surface proteins of N. meningitidis which are highly conserved across a plurality of strains, and which have immuno-protective profiles against N. meningitidis, and/or which may be used in combination with other components of N. meningitidis to enhance the efficacy of protection against this organism.

#### SUMMARY OF THE INVENTION

The present inventors have discovered a new gene which is present in all tested strains of N. meningitidis and which encodes a novel polypeptide having a predicted molecular weight of about 62 kDa. Based upon its sequence characteristics and homologies, this polypeptide is predicted to be an

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adhesin and this, together with experimental data suggests that it constitutes a surface protein which may be useful for the production of therapeutic and/or prophylactic vaccines against *N. meningitidis* as described hereinafter.

Accordingly, in one aspect of the invention, there is provided an isolated polypeptide or fragment thereof, or variant or derivative of these, said polypeptide selected from the group consisting of:

10 (a) a polypeptide according to SEQ ID NO 2;

- (b) a polypeptide according to SEQ ID NO 5;
- (c) a polypeptide according to SEQ ID NO 7;
- (d) a polypeptide according to SEQ ID NO 9;
- (e) a polypeptide according to SEQ ID NO 11;
- (f) a polypeptide according to SEQ ID NO 13;
- (g) a polypeptide according to SEQ ID NO 15;
- (h) a polypeptide according to SEQ ID NO 17;
- (i) a polypeptide according to SEQ ID NO 19; and
- (j) a polypeptide according to SEQ ID NO 21.

Preferably, said polypeptide, fragment, variant or derivative displays immunological activity against one or more members selected from the group consisting of:-

30 (i) N. meningitidis;

- (ii) said polypeptide;
- (iii) said fragment;
- (iv) said variant; and
- (v) said derivative;

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According to another aspect, the invention provides an isolated nucleic acid sequence encoding a polypeptide or fragment thereof, or variant or derivative of said fragment or polypeptide, according to the first-mentioned aspect. Suitably, said sequence is selected from the group consisting of:

- (1) the nucleotide sequence of SEQ ID NO 1;
- (2) the nucleotide sequence of SEQ ID NO 3;
- (3) the nucleotide sequence of SEQ ID NO 4;
- (4) the nucleotide sequence of SEQ ID NO 6;
- (5) the nucleotide sequence of SEQ ID NO 8;
- (6) the nucleotide sequence of SEQ ID NO 10;
- (7) the nucleotide sequence of SEQ ID NO 12;
- (8) the nucleotide sequence of SEQ ID NO 14;
- (9) the nucleotide sequence of SEQ ID NO 16;
- (10) the nucleotide sequence of SEQ ID NO 18;
- (11) the nucleotide sequence of SEQ ID NO 20;
- (12) a nucleotide sequence fragment of any one of SEQ ID NOS 1, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 20; and

(13) a nucleotide sequence homologue of any of the foregoing sequences

Preferably, said sequences encode a product displaying immunological activity against one or more members selected from the group consisting of:-

- (i) N. meningitidis;
- (ii) said polypeptide of the firstmentioned aspect;
- (iii) said fragment of said first-mentioned
   aspect;
- (iv) said variant of said first-mentioned
   aspect; and
- (v) said derivative of said firstmentioned aspect.

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In yet another aspect, the invention resides in an expression vector comprising a nucleic acid sequence according to the second-mentioned aspect wherein said sequence is operably linked to transcriptional and translational regulatory nucleic acid.

In a further aspect, the invention provides a host cell containing an expression vector according to the third-mentioned aspect.

In yet a further aspect of the invention, there is provided a method of producing a recombinant polypeptide according to the first-mentioned aspect, said method comprising the steps of:

- (A) culturing a host cell containing an expression vector according to the third-mentioned aspect such that said recombinant polypeptide is expressed from said nucleic acid; and
- (B) isolating said recombinant polypeptide.
- In a still further aspect, the invention provides an antibody or fragment thereof that binds to one or more members selected from the group consisting of:-
  - (1) N. meningitidis;
    - (2) said polypeptide of the first-mentioned aspect;
    - (3) said fragment of the first-mentioned aspect;
    - (4) said variant of the first-mentioned aspect; and
    - (5) said derivative of the first-mentioned aspect.

In yet another aspect, the invention provides a method of detecting N. meningitidis in a biological

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sample suspected of containing same, said method comprising the steps of:-

- (A) isolating the biological sample from a patient;
- (B) mixing the above-mentioned antibody or fragment with the biological sample to form a mixture; and
- (C) detecting specifically bound antibody or bound fragment in the mixture which indicates the presence of N. meningitidis.

According to a further aspect, there is provided a method of detecting N. meningitidis bacteria in a biological sample suspected of containing said bacteria, said method comprising the steps of:-

- (I) isolating the biological sample from a patient;
- (II) detecting a nucleic acid sequence according to the second-mentioned aspect in said sample which indicates the presence of said bacteria.

The invention further contemplates a method for diagnosing infection of patients by N. meningitidis, said method comprising the steps of:-

- (1) contacting a biological sample from a patient with a polypeptide, fragment, variant or derivative of the invention; and
- (2) determining the presence or absence of a complex between said polypeptide, fragment, variant or derivative and N. meningitidis-specific antibodies in said sample, wherein the presence of

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said complex is indicative of said infection.

The invention also extends to the use of the polypeptide according to the first-mentioned aspect, the use of the nucleic acids according to the second-mentioned aspect or the use of the antibody or antibody fragment mentioned above in a kit for detecting N. meningitidis bacteria in a biological sample.

According to a further aspect of the invention, there is provided a pharmaceutical composition comprising an isolated polypeptide or fragment thereof, or a variant or derivative of these, according to the first mentioned aspect.

Preferably, said pharmaceutical composition is a vaccine.

In yet a further aspect, the invention provides a method of preventing infection of a patient by N. meningitidis, comprising the step of administrating a pharmaceutically effective amount of the above-mentioned vaccine.

In a further aspect, the invention provides a method of identifying an immunoreactive fragment of a polypeptide, variant or derivatives according to the first mentioned aspect, comprising the steps of:-

- (a) generating a fragment of said polypeptide, variant or derivative;
- (b) administering said fragment to a mammal; and
- (c) detecting an immune response in said mammal which response includes production of elements which specifically bind N. meningitidis and/or said polypeptide, variant or

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derivative, and/or a protective effect against N. meningitidis infection.

# BRIEF DESCRIPTION OF THE DRAWINGS

"FIG. 1 depicts plasmid maps and cloning strategy. Primers A3A and A3B (SEQ ID NOS 28 and 29, respectively) were used to amplify from MC58 the region identified in the TIGR database as a homologue of AIDA-I". PCR product was cloned to give pNMAIDA3. Primers A3C (SEQ ID NO 30) and A3D (SEQ ID NO 31) were used in inverse PCR to amplify a 3kbp EagI fragment encompassing hiaNm. This product was cloned to give piEAGA3 was subcloned to give piEagA3.8 and Primers HiaNm:M and HiaNm:P (SEQ ID NOS 22 piEagA3.9. and 23, respectively) were used to amplify the contiquous region from MC58 and the product cloned to Primers Hia-MBPA (SEQ ID NO 24) and create pHiaNm. Hia-MBPB (SEQ ID NO 25) were used to amplify the open reading frame of hiaNm, and the product was cloned into pMALC2 to create pMBP-HiaNm;

FIG. 2 is a Southern blot of genomic DNA of a number of strains of N. meningitidis. 2A: serogroup B strains. Lane 1 PMC28, Lane 2 PMC27, Lane 3 PMC25, Lane 4 PMC24, Lane 5 PMC16, Lane 6 PMC13, Lane 7 PMC12, Lane 8 MWt standards, Lane 9 2970, Lane 10 1000, Lane 11 528 Lane 12 SWZ107, Lane 13 H41, Lane 14 H38, Lane 15 NGH36, Lane 16 H15, Lane 17 NGG40, Lane 18 NGF26, Lane 19 NGE30, Lane 20 Lane NGE28 2B: Strains of serogroups other than B. Lane 1 PMC3, Lane 2 PMC17, Lane 3 PMC20, Lane 4 PMC23, Lane 5 PMC8, Lane 6 PMC9, Lane 7 PMC11, Lane 8 PMC14, Lane 9 PMC18, Lane 10 PMC21, Lane 11 PMC29, Lane 12 MWt standards, Lane 13 PMC19, Lane 14 PMC1, Lane 15 PMC6, Lane 16 PMC10, Lane 17 PMC22, Lane 18 PMC26, Lane 19 PMC2. Molecular

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weight markers indicated in kilobase pairs (kb). Genomic DNA was hybridized with a probe corresponding to ntp 276-2054 of SEQ ID NO 1;

FIG. 3 shows a Coomassie stained gel of MBP-HiaNm. Cells containing pMALC2 (Lane 2) or pMBP-HiaNm (Lane 3) after induction with IPTG. Lane 1 molecular weight standards (kDa). Arrows indicate MBP and MBP-HiaNm;

FIG. 4 is a western blot of MC58 and MC58ΔHiaNm proteins incubated with rabbit immune sera. Lane 1; molecular weight standards indicated in kDa, Lane 2 total cellular protein of MC58, Lane 3 total cellular protein of MC58ΔHiaNm Lane 4, OMC preparation of MC58, Lane 5 OMC preparation of MC58ΔHiaNm, each lane contained 50 μL of protein suspension of A<sub>280</sub>= 3.75;

FIG. 5 shows a Coomassie stained gel run in parallel to the gel that was Western blotted in FIG 4. Lanes are the same as for FIG 4;

FIG. 6 shows a sequence comparison of polypeptides of HiaNm, Hia, Hsf using the PILEUP alignment program; and

FIG. 7 shows a sequence comparison of polypeptide sequences of HiaNm from 10 strains of N. meningitidis using the PILEUP program

## DETAILED DESCRIPTION OF THE INVENTION

specification and the Throughout this appendant claims, unless the context requires "comprise", "comprises" the words otherwise, "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the rexclusion of any other integer or group of integers.

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### Polypeptide sequences

The present invention provides an isolated polypeptide according to SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21, or fragment respectively thereof, or variant or derivative of these. In a preferred embodiment, the polypeptide, fragments, variants and derivatives of the invention display immunological activity against any one member selected from the group consisting of N. meningitidis, said polypeptide, said fragment, said variant and said derivative.

SEQ ID NO 2 corresponds to the novel about 62 kDa surface polypeptide of the hiaNm gene obtained from N. meningitidis strain MC58, as described more fully hereinafter. SEQ ID NOS 5, 7, 9, 11, 13, 15, 17, 19, and 21 correspond to homologous polypeptides deduced from nucleotide sequences obtained from N. meningitidis strains BZ10, BZ198, EG327, EG329, H15, H38, H41, P20, and PMC21, respectively.

For the purposes of this invention, the term "immunological activity" refers to the ability of the aforementioned polypeptide, fragment, variant or derivative to produce an immune response in a mammal to which it is administered, wherein the response includes the production of elements which specifically bind N. meningitidis and/or said polypeptide, fragment, variant or derivative, and/or a protective effect against N. meningitidis infection.

By "isolated" is meant material which is substantially or essentially free from components which normally accompany it in its native state.

By "polypeptide" is meant long chain peptides including proteins.

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As used herein, the term "fragment" includes deletion mutants and small peptides, for example of at least 6, preferably at least 10 and more preferably at length, which comprise amino acids in antigenic determinants or epitopes. Several such Peptides of this fragments may be joined together. type may be obtained through the application of techniques nucleic acid recombinant standard synthesized using conventional liquid or solid phase For example, reference may be synthesis techniques. made to solution synthesis or solid phase synthesis as described, for example, in Chapter 9 entitled "Peptide Synthesis" by Atherton and Shephard which is included in a publication entitled "Synthetic Vaccines" edited by Nicholson and published by Blackwell Scientific Alternatively, peptides can be produced Publications. by digestion of a polypeptide of the invention with proteinases such as endoLys-C, endoArg-C, endoGlu-C digested The staphylococcins V8-protease. and fragments can be purified by, for example, performance liquid chromatographic (HPLC) techniques.

The term "variant" refers to polypeptides in which one or more amino acids have been replaced by different amino acids. It is well understood in the art that some amino acids may be changed to others with broadly similar properties without changing the polypeptide of the activity the nature of Exemplary conservative (conservative substitutions). substitutions in the polypeptide may be made according to the following table:

TABLE 1

Original Residue	Exemplary Substitutions		
Ala	Ser		

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•	<u> </u>
Arg	, Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile,
Phe	Met, Leu, Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu
	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1

Substantial changes in function are made by selecting substitutions that are less conservative than those shown in TABLE 1. Other replacements would be non-conservative substitutions and relatively fewer Generally, tolerated. of these be substitutions which are likely to produce the greatest changes in a polypeptide's properties are those in which (a) a hydrophilic residue (e.g., Ser or Thr) is substituted for, or by, a hydrophobic residue (e.g., Ala, Leu, Ile, Phe or Val); (b) a cysteine or proline is substituted for, or by, any other residue; (c) a residue having an electropositive side chain (e.g., substituted for, for by, is Arg, His or Lys) electronegative residue (e.g., Glu or Asp) or (d) a residue having a bulky side chain (e.g., Phe or Trp) is substituted for, or by, one having a smaller side chain (e.g., Ala, Ser) or no side chain (e.g., Gly).

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In general, variants will be at least 75% homologous, more suitably at least 80%, preferably at least 85%, and most preferably at least 90% homologous to the basic sequences as for example shown in SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21. Homology is defined as the percentage number of amino acids identical or constitute conservative substitutions as defined in Table 1. Homology may be determined using sequence comparison programs such as GAP (Deveraux et al. 1984, Nucleic Acids Research 12, 387-395) which is incorporated herein by reference. In this way sequences of a similar or substantially different length to those cited herein may be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP. What constitutes suitable variants may be determined by conventional techniques. nucleic acids encoding polypeptides For example, according to SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21 can be mutated using either random mutagenesis for example using transposon mutagenesis, or sitedirected mutagenesis. The resultant DNA fragments are then cloned into suitable expression hosts such as E. coli using conventional technology and clones which retain the desired activity are detected. Where the clones have been derived using random mutagenesis techniques, positive clones would have to be sequenced in order to detect the mutation. The term "variant" also includes naturally occurring allelic variants.

By "derivative" is meant a polypeptide which has been derived from the basic sequence by modification, for example by conjugation or complexing with other chemical moieties or by post-translational modification techniques as would be understood in the

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art. Such derivatives include amino acid deletions and/or additions to polypeptides according to SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21 or variants thereof wherein said derivatives retain immunological "Additions" of amino acids may include activity. fusion of the polypeptides or variants thereof with other polypeptides or proteins. In this regard, it will be appreciated that the polypeptides or variants of the invention may be incorporated into larger polypeptides, and such larger polypeptides may also be expected to retain immunological activity against, for The polypeptides meningitidis. example, N. described above may be fused to a further protein, for example, which is not derived from N. meningitidis. The other protein may, by way of example, assist in the purification of the protein. For instance a polyhistidine tag, or a maltose binding protein may be used in this respect as described in more detail Alternatively, it may produce an immune below. response which is effective against N. meningitidis or it may produce an immune response against another Other possible fusion proteins are those pathogen. response. produce immunomodulatory an which Particular examples of such proteins include Protein A or glutathione S-transferase (GST). In addition, the polypeptide may be fused to an oligosaccharide based vaccine component where it acts as a carrier protein.

the derivatives contemplated by Other are not limited to, include, but invention incorporation side chains, modification to unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use methods which crosslinkers and other the polypeptides, constraints on conformational fragments and variants of the invention.

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Examples of side chain modifications invention contemplated by the present include modifications of amino groups such as by acylation with acetic anhydride; acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; amidination with methylacetimidate; carbamoylation of amino groups with cyanate; pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH4; reductive alkylation by reaction with reduction with NaBH<sub>4</sub>; and followed by aldehyde trinitrobenzylation of amino groups with 2, 4, 6trinitrobenzene sulphonic acid (TNBS).

The carboxyl group may be modified by carbodimide activation via 0-acylisourea formation followed by subsequent derivitization, by way of example, to a corresponding amide.

The guanidine group of arginine residues may be modified by formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

Sulphydryl groups may be modified by methods such as performic acid oxidation to cysteic acid; 4using mercurial derivatives formation of acid, 4chloromercuriphenylsulphonic chloromercuribenzoate; 2-chloromercuri-4-nitrophenol, mercurials; other and phenylmercury chloride, formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; carboxymethylation iodoacetamide; and acid or iodoacetic with carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified, for example, by alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphonyl halides or by oxidation with N-bromosuccinimide.

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Tyrosine residues, may be modified by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

The imidazole ring of a histidine residue may be modified by N-carbethoxylation with diethylpyrocarbonate or by alkylation with iodoacetic acid derivatives.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include but are not limited to, use of 4-amino butyric acid, 4-amino-3-hydroxy-5acid, 6-aminohexanoic 4-amino-3-hydroxy-6phenylpentanoic acid, acid, t-butylglycine, norleucine, methylheptanoic norvaline, phenylglycine, ornithine, sarcosine, thienyl alanine and/or D-isomers of amino acids. list of unnatural amino acids contemplated by the present invention is shown in TABLE 2.

TABLE 2

Non-conventional amino acid	Non-conventional amino acid
α-aminobutyric acid	L-N-methylalanine
α-amino-α-methylbutyrate	L-N-methylarginine
aminocyclopropane-carboxylate	L-N-methylasparagine
aminoisobutyric acid	L-N-methylaspartic acid
aminonorbornyl-carboxylate	L-N-methylcysteine
cyclohexylalanine	L-N-methylglutamine
cyclopentylalanine	L-N-methylglutamic acid
L-N-methylisoleucine	L-N-methylhistidine
D-alanine	L-N-methylleucine
D-arginine	L-N-methyllysine
D-aspartic acid	L-N-methylmethionine
D-cysteine	L-N-methylnorleucine
D-glutamate	L-N-methylnorvaline
D-glutamic acid	L-N-methylornithine
D-histidine	L-N-methylphenylalanine
D-isoleucine	L-N-methylproline
D-leucine	L-N-medlylserine

D-lysine L-N-methylthreonine D-methionine L-N-methyltryptophan D-ornithine L-N-methyltyrosine L-N-methylvaline D-phenylalanine L-N-methylethylglycine D-proline L-N-methyl-t-butylglycine D-serine D-threonine L-norleucine D-tryptophan L-norvaline D-tyrosine α-methyl-aminoisobutyrate D-valine α-methyl-γ-aminobutyrate D-α-methylalanine α-methylcyclohexylalanine α-methylcylcopentylalanine D-α-methylarginine  $\alpha$ -methyl- $\alpha$ -napthylalanine D-α-methylasparagine α-methylpenicillamine D-α-methylaspartate N-(4-aminobutyl)glycine D-α-methylcysteine N-(2-aminoethyl)glycine D-α-methylglutamine N-(3-aminopropyl)glycine D-α-methylhistidine N-amino-α-methylbutyrate D-α-methylisoleucine α-napthylalanine D-α-methylleucine N-benzylglycine D-α-methyllysine N-(2-carbamylediyl)glycine D-α-methylmethionine N-(carbamylmethyl)glycine D-α-methylornithiine N-(2-carboxyethyl)glycine D-α-methylphenylalanine N-(carboxymethyl)glycine D-α-methylproline N-cyclobutylglycine  $D-\alpha$ -methylserine N-cycloheptylglycine D-α-methylthreonine N-cyclohexylglycine D-α-methyltryptophan N-cyclodecylglycine D-α-methyltyrosine L-α-methyllysine L-α-methylleucine L-a-methylnorleucine L-α-methylmethionine L-α-methylornithine L-α-methylnorvatine  $L-\alpha$ -methylproline L-α-methylphenylalanine L-a-methylthreonine L-a-methylserine L-α-methyltyrosine L-α-methyltryptophan L-N-methylhomophenylalanine  $L-\alpha$ -methylvaline N-(N-(2,2-diphenylethyl N-(N-(3,3-diphenylpropyl

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 $\langle \gamma_i \rangle \langle \gamma_i \rangle$ 

carbamylmethyl)glycine	carbamylmethyl)glycine
1-carboxy-1-(2,2-diphenyl-ethyl	
amino) cyclopropane	

The invention also contemplates covalently modifying a polypeptide, fragment or variant of the invention with dinitrophenol, in order to render it immunogenic in humans

Preferably the invention comprises a polypeptide selected from any one of the polypeptides according to SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21.

Polypeptides of the inventions may be prepared by any suitable procedure known to those of skill in the art. For example, the polypeptides may be prepared by a procedure including the steps of:

- (a) preparing a recombinant nucleic acid containing a nucleotide sequence encoding a polypeptide according to any one of SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21, or fragment thereof, or variant or derivative of these, which nucleotide sequence is operably linked to transcriptional and translational regulatory nucleic acid;
- (b) transfecting or transforming a suitable host cell with the recombinant nucleic acid;
- (c) culturing the host cell to express recombinant polypeptide from said recombinant nucleic acid; and
  - (d) isolating the recombinant polypeptide.

Suitably said nucleotide sequence is selected from the group consisting of SEQ ID NOS 1, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 20.

By "recombinant polypeptide" is meant a polypeptide made using recombinant techniques, i.e., through the expression of a recombinant nucleic acid.

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The term "recombinant nucleic acid" as used herein refers to nucleic acid formed in vitro by the manipulation of nucleic acid into a form not normally In this regard, the recombinant found in nature. nucleic acid preferably comprises an expression vector either a self-replicating may be chromosomal vector such as a plasmid, or a vector which integrates into a host genome. Generally, such include transcriptional expression vectors translational regulatory nucleic acid operably linked to the said nucleotide sequence.

By "operably linked" is meant that the transcriptional and translational regulatory nucleic acid is positioned relative to the nucleotide sequence encoding the said polypeptide, fragment, variant or derivative in such a manner that such transcription is The transcriptional and translational initiatable. regulatory nucleic acid will generally be appropriate for the host cell used for expression. Numerous types suitable expression vectors, and appropriate regulatory sequences are known in the for a art variety of host cells.

transcriptional and Typically, the translational regulatory nucleic acid may include, but is not limited to, promoter sequences, leader or sequences, ribosomal binding sites, signal sequences, start and stop transcriptional translational start and stop sequences, and enhancer or activator sequences.

Constitutive or inducible promoters as known in the art are contemplated by the invention. The promoters may be either naturally occurring promoters, or hybrid promoters which combine elements of more than one promoter.

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In a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

The expression vector may also include a fusion partner (typically provided by the expression vector) so that the recombinant polypeptide of the invention is expressed as a fusion polypeptide with said fusion partner. The main advantage of fusion partners is that they assist identification and/or purification of said fusion polypeptide.

In order to express said fusion polypeptide, it is necessary to ligate a nucleotide sequence according to the invention into the expression vector so that the translational reading frames of the fusion partner and the nucleotide sequence of the invention coincide.

known examples of fusion partners Well not limited to, glutathione-Sare include, but transferase (GST), Fc potion of human IgG, maltose binding protein (MBP) and hexahistidine (HIS6), which are particularly useful for isolation of the fusion polypeptide by affinity chromatography. For the fusion polypeptide purification purposes of relevant matrices affinity chromatography, affinity chromatography are glutathione-, amylose-, and nickel- or cobalt-conjugated resins respectively. Many such matrices are available in "kit" form, such as the QIAexpress™ system (Qiagen) useful with (HIS<sub>6</sub>) fusion partners and the Pharmacia GST purification system.

Another fusion partner well known in the art is green fluorescent protein (GFP). This fusion partner serves as a fluorescent "tag" which allows the

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fusion polypeptide of the invention to be identified by fluorescence microscopy or by flow cytometry. useful when is assessing subcellular tag localization fusion polypeptide - of of the invention, or for isolating cells which express the fusion polypeptide of the invention. Flow cytometric methods such as fluorescence activated cell sorting this latter (FACS) are particularly useful in application.

Preferably, the fusion partners also have protease cleavage sites, such as for Factor Xa or Thrombin, which allow the relevant protease partially digest the fusion polypeptide of the the recombinant liberate and thereby polypeptide of the invention therefrom. The liberated 15 polypeptide can then be isolated from the fusion partner by subsequent chromatographic separation.

Fusion partners according to the invention also include within their scope "epitope tags", which are usually short peptide sequences for which a Well known examples specific antibody is available. for which specific monoclonal epitope tags include C-myc, readily available antibodies are influenza virus haemagglutinin and FLAG tags.

Recombinant polypeptides of the invention may be produced by culturing a host cell transformed with an expression vector containing nucleic acid fragment, variant encoding polypeptide, а derivative according to the invention. The conditions appropriate for protein expression will vary with the choice of expression vector and the host cell. This is easily ascertained by one skilled in the art through routine experimentation.

Suitable host cells for expression may be prokaryotic or eukaryotic. One preferred host cell

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for expression of a polypeptide according to the invention is a bacterium. The bacterium used may be *Escherichia coli*. Alternatively, the host cell may be an insect cell such as, for example, *SF9* cells which may be utilized with a baculovirus expression system.

The recombinant protein may be conveniently prepared by a person skilled in the art using standard protocols as for example described in Sambrook, et al., MOLECULAR CLONING. A LABORATORY MANUAL (Cold Spring Harbor Press, 1989), incorporated herein by reference, in particular Sections 16 and 17; Ausubel et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (John Wiley & Sons, Inc. 1994-1998), incorporated herein by reference, in particular Chapters 10 and 16; and Coligan et al., CURRENT PROTOCOLS IN PROTEIN SCIENCE, (John Wiley & Sons, Inc. 1995-1997) which is incorporated by reference herein, in particular Chapters 1, 5 and 6.

### Nucleotide sequences

The invention further provides a nucleotide sequence which encodes a polypeptide, fragment, variant or derivative as defined above. Suitably said sequence is selected from the group consisting of:—SEQ ID NOS 1, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 20; a nucleotide sequence fragment of any one of SEQ ID NOS 1, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 20; and a nucleotide sequence homologue of the foregoing sequences. Preferably, these sequences encode a product displaying immunological activity as defined above.

As will be more fully described hereinafter,
SEQ ID NO 1 corresponds to the hiaNm gene obtained
from N. meningitidis strain MC58. This gene encodes

the novel 62 kDa (approximately) surface polypeptide of SEQ ID NO 2. SEQ ID NO 3 corresponds to the hiaNm open reading frame sequence of strain MC58, HiaNm. SEQ ID NOS 4, 6, 8, 10, 12, 14, 16, 18, and 20 correspond to the homologous hiaNm open reading frame sequences obtained from N. meningitidis strains BZ10, BZ198, EG327, EG329, H15, H38, H41, P20, and PMC21, respectively.

The term "nucleotide sequence" as used 10 herein designates mRNA, RNA, cRNA, cDNA or DNA.

The term "nucleotide sequence homologues" nucleotide sequences which to refers generally sequence wild-type nucleotide hybridize with a under substantially invention the according to stringent conditions. Suitable hybridization conditions will be discussed hereinafter.

The nucleotide sequence homologues of the invention may be prepared according to the following procedure:

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- (i) obtaining a nucleic acid extract from a suitable host;
- (ii) creating primers which are optionally degenerate wherein each comprises a portion of a wild-type nucleotide sequence of the invention; and

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(iii) using said primers to amplify, via nucleic acid amplification techniques, one or more amplification products from said nucleic acid extract.

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Suitably, the host may be a bacterium. Preferably, the host is from the genus Neisseria, more preferably from N. meningitidis.

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Preferably, the primers are selected from the group consisting of:-

- (1) 5'-TTAGATTCCACGTCCCAGATT-3' (SEQ ID NO 22);
- (2) 5'-CTTCCCTTCAAACCTTCC-3' (SEQ ID NO 23);
- (3) 5'-GGTCGCGGATCCATGAACAAATATACCGCAT-3'
  (SEQ ID NO 24);
- (4) 5'-TCACCCAAGCTTAAGCCCTTACCACTGATAAC-3' (SEO ID NO 25);
- (5) 5'-CCAAACCCCGATTTAACC-3' (SEQ ID NO 26);
- (6) 5'-AATCGCCACCCTTCCCTTC-3' (SEQ ID NO 27);
- (7) 5'-TTTGCAACGGTTCAGGCA-3' (SEQ ID NO 28);
- (8) 5'-TATTCAGCAGCGTATCGG-3' (SEQ ID NO 29);
- (9) 5'-TGCCTGAACCGTTGCAAA-3' (SEQ ID NO 30); and
- (10) 5'-CCGATACGCTGCTGAATA-3' (SEQ ID NO 31).

amplification acid Suitable nucleic techniques are well known to the skilled addressee, and include polymerase chain reaction (PCR) as for example described in Ausubel et al. (1994-1998, supra, Chapter 15) which is incorporated herein by reference; strand displacement amplification (SDA) as for example 5,422,252 which described in U.S. Patent No reference; rolling circle incorporated herein by replication (RCR) as for example described in Liu et Chem. Soc. 118:1587-1594 and al., (1996, J. Am. International application WO 92/01813) and Lizardi et al., (International Application WO 97/19193) which are

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incorporated herein by reference; nucleic acid sequence-based amplification (NASBA) as for example described by Sooknanan et al., (1994, Biotechniques 17:1077-1080) which is incorporated herein by reference; and Q- $\beta$  replicase amplification as for example described by Tyagi et al., (1996, Proc. Natl. Acad. Sci. USA 93:5395-5400) which is incorporated herein by reference.

As used herein, an "amplification product"

refers to a nucleic acid product generated by nucleic acid amplification techniques.

"Hybridize" or "hybridization" is used here to denote the pairing of complementary bases of distinct nucleotide sequences to produce a DNA-DNA hybrid, a DNA-RNA hybrid, or an RNA-RNA hybrid according to base-pairing rules.

In DNA, complementary bases are:

- (i) A and T; and
- (ii) C and G.

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- (i) A and U; and
- (ii) C and G.

In RNA-DNA hybrids, complementary bases are:

- (i) A and U;
- (ii) A and T; and
- (iii) G and C.

substantially 🤲 complementary Typically, identified by blotting are nucleotide sequences techniques that include a step whereby nucleotides are (preferably a synthetic immobilized on a matrix hybridization membrane such as nitrocellulose), a step, and a detection step. Southern blotting is used to identify a complementary DNA sequence; northern blotting is used to identify a complementary RNA

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sequence. Dot blotting and slot blotting can be used to identify complementary DNA/DNA, DNA/RNA or RNA/RNA polynucleotide sequences. Such techniques are well known by those skilled in the art, and have been described in Ausubel et al. (1994-1998, supra) at pages 2.9.1 through 2.9.20.

According to such methods, Southern blotting involves separating DNA molecules according to size by gel electrophoresis, transferring the size-separated DNA to a synthetic membrane, and hybridizing the membrane bound DNA to a complementary nucleotide sequence labeled radioactively, enzymatically or fluorochromatically. In dot blotting and slot blotting, DNA samples are directly applied to a synthetic membrane prior to hybridization as above.

An alternative blotting step is used when identifying complementary nucleotide sequences in a cDNA or genomic DNA library, such as through the process of plaque or colony hybridization. A typical example of this procedure is described in Sambrook et al., (1989, supra) Chapters 8-12.

Typically, the following general procedure can be used to determine hybridization conditions. Nucleotide sequences are blotted/transferred to a synthetic membrane, as described above. A wild type nucleotide sequence of the invention is labeled as described above, and the ability of this labeled nucleotide sequence to hybridize with an immobilized nucleotide sequence analyzed.

A skilled addressee will recognize that a number of factors influence hybridization. The specific activity of radioactively labeled polynucleotide sequence should typically be greater than or equal to about 10<sup>8</sup> dpm/mg to provide a detectable signal. A radiolabeled nucleotide sequence

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of specific activity 10<sup>8</sup> to 10<sup>9</sup> dpm/mg can detect approximately 0.5 pg of DNA. It is well known in the art that sufficient DNA must be immobilized on the membrane to permit detection. It is desirable to have excess immobilized DNA, usually 10µg. Adding an inert polymer such as 10% (w/v) dextran sulfate (MW 500,000) or polyethylene glycol 6000 during hybridization can also increase the sensitivity of hybridization (see Ausubel supra at 2.10.10).

from meaningful results To achieve nucleotide sequence hybridization between а immobilized on a membrane and a labeled nucleotide labeled amount of the sufficient sequence, must be hybridized to the nucleotide sequence following washing. immobilized nucleotide sequence Washing ensures that the labeled nucleotide sequence is hybridized only to the immobilized nucleotide sequences with a desired degree of complementarity to the labeled nucleotide sequence.

"Stringency" as used herein, refers to the temperature and ionic strength conditions, and presence or absence of certain organic solvents, during hybridization. The higher the stringency, the higher will be the degree of complementarity between the immobilized nucleotide sequences and the labeled polynucleotide sequence.

"Stringent conditions" designates those conditions under which only nucleotide sequences having a high frequency of complementary bases will hybridize.

Typical stringent conditions include, for example, (1) 0.75 M dibasic sodium phosphate/0.5 M monobasic sodium phosphate/1 mM disodium EDTA/1% sarkosyl at about 42°C for at least 30 minutes; or (2)

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6.0 M urea/0.4 % sodium lauryl sulfate/0.1x SSC at about 42°C for at least 30 minutes; or (3) 0.1x SSC/0.1% SDS at about 68°C for at least 20 minutes; or (4) 1x SSC/0.1% SDS at about 55°C for about 60 minutes; or (5) 1x SSC/0.1% SDS at about 62°C for about 60 minutes; or (6) 1x SSC/0.1% SDS at about 68°C for about 60 minutes; or (7) 0.2X SSC/0.1% SDS at about 55°C for about 60 minutes; or (8) 0.2x SSC/0.1% SDS at about 62°C for about one hour; or (9) 0.2X SSC/0.1% SDS at about 68°C for about 60 minutes. For a detailed example, see CURRENT PROTOCOLS IN MOLECULAR BIOLOGY supra at pages 2.10.1 to 2.10.16, and Sambrook et al. in MOLECULAR CLONING. A LABORATORY MANUAL (Cold Spring Harbour Press, 1989) at sections 1.101 to 1.104, which are hereby incorporated by reference.

While stringent washes are typically carried out at temperatures from about 42°C to 68°C, that skilled in the art will appreciate temperatures may be suitable for stringent conditions. Maximum hybridization typically occurs at about 20°C to 25°C below the  $T_m$  for formation of a DNA-DNA hybrid. It is well known in the art that the  $T_m$  is the melting temperature, or temperature at which two complementary polynucleotide sequences dissociate. Methods for estimating  $T_m$  are well known in the art (see CURRENT PROTOCOLS IN MOLECULAR BIOLOGY supra at page 2.10.8). Maximum hybridization typically occurs at about 10°C to 15°C below the T<sub>m</sub> for a DNA-RNA hybrid.

Other stringent conditions are well-known in the art. A skilled addressee will recognize that various factors can be manipulated to optimize the specificity of the hybridization. Optimization of the stringency of the final washes can serve to ensure a high degree of hybridization.

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Methods for detecting labeled nucleotide sequences hybridized to an immobilized nucleotide sequence are well known to practitioners in the art. Such methods include autoradiography, chemiluminescent, fluorescent and colorimetric detection.

#### Antibodies

The invention also contemplates antibodies against the aforementioned polypeptides, fragments, variants and derivatives. Such antibodies may include any suitable antibodies which bind to or conjugate with a polypeptide, fragment, variant or derivative of For example, the antibodies may invention. comprise polyclonal antibodies. Such antibodies may be prepared for example by injecting a polypeptide, fragment, variant or derivative of the invention into a production species, which may include mice or Methods of rabbits, to obtain polyclonal antisera. producing polyclonal antibodies are well known those skilled in the art. Exemplary protocols which may be used are described for example in Coligan et al., CURRENT PROTOCOLS IN IMMUNOLOGY, (John Wiley & Sons, Inc, 1991) which is incorporated herein by reference, and Ausubel et al., (1994-1998, supra), in particular Section III of Chapter 11.

In lieu of the polyclonal antisera obtained in the production species, monoclonal antibodies may be produced using the standard method as for example, described in an article by Köhler and Milstein (1975, Nature 256, 495-497) which is herein incorporated by reference, or by more recent modifications thereof as for example, described in Coligan et al., (1991, supra) by immortalizing spleen or other antibody

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producing cells derived from a production species which has been inoculated with one or more of the polypeptides, fragments, variants or derivatives of the invention.

The invention also includes within its scope antibodies which comprise Fc or Fab fragments of the polyclonal or monoclonal antibodies referred to above. Alternatively, the antibodies may comprise single chain Fv antibodies (scFvs) against the peptides of the invention. Such scFvs may be prepared, for example, in accordance with the methods described respectively in United States Patent No 5,091,513, European Patent No 239,400 or the article by Winter and Milstein (1991, Nature, 349 293) which are incorporated herein by reference.

The antibodies of the invention may be used for affinity chromatography in isolating natural or recombinant N. meningitidis polypeptides. For example reference may be made to immunoaffinity chromatographic procedures described in Chapter 9.5 of Coligan et al., (1995-1997, supra).

The antibodies can be used to screen expression libraries for variant polypeptides of the invention. The antibodies of the invention can also be used to detect N. meningitidis infection described hereinafter.

# Detection of N. meningitidis

The presence or absence of N. meningitidis in a patient may determined by isolating a biological sample from a patient, mixing an antibody or antibody fragment described above with the biological sample to form a mixture, and detecting specifically bound antibody or bound fragment in the mixture which

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indicates the presence of N. meningitidis in the sample.

The term "biological sample" as used herein refers to a sample which may be extracted, untreated, 5 treated, diluted or concentrated from a patient. Suitably, the biological sample is selected from the group consisting of whole blood, serum, saliva, urine, sweat, ascitic fluid, peritoneal fluid, synovial fluid, amniotic fluid, cerebrospinal fluid, skin biopsy, and the like.

technique for determining suitable Any formation of the complex may be used. For example, an antibody or antibody fragment according to the invention having a label associated therewith may be Such immunoassays may utilized in immunoassays. include, but are not limited to, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs) and immunochromatographic techniques (ICTs) which are well known those of skill in the art. For example, reference may be made to "CURRENT PROTOCOLS IMMUNOLOGY" (1994, supra) which discloses a variety of immunoassays that may be used in accordance with the Immunoassays may include invention. present competitive assays as understood in the art.

The label associated with the antibody or antibody fragment may include the following:

- direct attachment of the label to the i. antibody or antibody fragment;
- indirect attachment of the label to the ii. antibody or antibody fragment; i.e., attachment of the label to another assay reagent which subsequently binds to the antibody or antibody fragment; and

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iii. attachment to a subsequent reaction product of the antibody or antibody fragment.

The label may be selected from a group including a chromogen, a catalyst, an enzyme, a fluorophore, a chemiluminescent molecule, a lanthanide ion such as Europium (Eu<sup>34</sup>), a radioisotope and a direct visual label.

In the case of a direct visual label, use may be made of a colloidal metallic or non-metallic particle, a dye particle, an enzyme or a substrate, an organic polymer, a latex particle, a liposome, or other vesicle containing a signal producing substance and the like.

A large number of enzymes suitable for use disclosed in United States Patent is Specifications U.S. 4,366,241, U.S. 4,843,000, U.S. 4,849,338, all of which are herein incorporated Suitable enzyme labels useful in the by reference. include alkaline invention phosphatase, present horseradish peroxidase, luciferase,  $\beta$ -galactosidase, glucose oxidase, lysozyme, malate dehydrogenase and The enzyme label may be used alone or in the like. combination with a second enzyme which is in solution.

Suitably, the fluorophore is selected from a group including fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate (TRITL) or R-Phycoerythrin (RPE).

The invention also extends to a method for detecting infection of patients by *N. meningitidis*, said method comprising the steps of contacting a biological sample from a patient with a polypeptide, fragment, variant or derivative of the invention, and determining the presence or absence of a complex

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between said polypeptide, fragment, variant or derivative and *N. meningitidis*-specific antibodies in said serum, wherein the presence of said complex is indicative of said infection.

In a preferred embodiment, detection of the above complex is effected by detectably modifying said polypeptide, fragment, variant or derivative with a suitable label as is well known in the art and using such modified compound in a suitable immunoassay as for example described above.

In another aspect, the invention provides a method of detecting N. meningitidis bacteria in a biological sample suspected of containing said bacteria, said method comprising the steps of isolating the biological sample from a patient, detecting a nucleic acid sequence according to the invention in said sample which indicates the presence of said bacteria.

Detection of the said nucleic acid sequence may be determined using any suitable technique. example, a labeled nucleic acid sequence according to the invention may be used as a probe in a Southern blot of a nucleic acid extract obtained from a patient as is well known in the art. Alternatively, a labeled nucleic acid sequence according to the invention may be utilized as a probe in a Northern blot of a RNA extract from the patient. Preferably, a nucleic acid extract from the patient is utilized in concert with oligonucleotide primers corresponding to sense and nucleic acid sequence sequences of a antisense according to the invention, or flanking sequences thereof, in a nucleic acid amplification reaction such as PCR, or the ligase chain reaction (LCR) as for International Application described in example WO89/09385 which is incorporated by reference herein.

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A variety of automated solid-phase detection techniques are also appropriate. For example, very large scale immobilized primer arrays (VLSIPS<sup>TM</sup>) are used for the detection of nucleic acids as for example described by Fodor et al., (1991, Science 251:767-777) and Kazal et al., (1996, Nature Medicine 2:753-759). The above generic techniques are well known to persons skilled in the art.

#### Pharmaceutical compositions

A further feature of the invention is the of polypeptide, fragment, variant the derivative of the invention ("immunogenic agents") as actives in a pharmaceutical composition for protecting N. meningitidis. by against infection patients Suitably, the pharmaceutical composition comprises a pharmaceutically-acceptable carrier.

By "pharmaceutically-acceptable carrier" is liquid filler, diluent solid or meant encapsulating substance which may be safely used in Depending upon the administration. systemic particular route of administration, a variety pharmaceutically-acceptable carriers, well known These carriers may be selected the art may be used. from a group including sugars, starches, cellulose and derivatives, malt, gelatine, talc, calcium its polyols, sulfate, vegetable oils, synthetic oils, buffered solutions, phosphate acid, alginic emulsifiers, isotonic saline, and pyrogen-free water.

Any suitable route of administration may be employed for providing a patient with the composition of the invention. For example, oral, rectal, parenteral, sublingual, buccal, intravenous, intraarticular, intra-muscular, intra-dermal, subcutaneous,

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inhalational, intraocular, intraperitoneal, intracerebroventricular, transdermal and the like may be employed. Intra-muscular and subcutaneous injection is appropriate, for example, for administration of immunogenic compositions, vaccines and DNA vaccines.

Dosage forms include tablets, dispersions, suspensions, injections, solutions, syrups, troches, capsules, suppositories, aerosols, transdermal patches These dosage forms may also include and the like. injecting or implanting controlled releasing devices designed specifically for this purpose or other forms of implants modified to act additionally in this Controlled release of the therapeutic agent may be effected by coating the same, for example, with hydrophobic polymers including acrylic resins, waxes, higher aliphatic alcohols, polylactic and polyglycolic and certain cellulose derivatives such acids In addition, hydroxypropylmethyl cellulose. controlled release may be effected by using other polymer matrices, liposomes and/or microspheres.

Pharmaceutical compositions of the present or parenteral suitable for oral invention administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of one or more therapeutic agents of the invention, as a powder or granules or as a solution or a suspension in an aqueous liquid, a nonaqueous liquid, an oil-in-water emulsion or a waterin-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association one or more immunogenic agents as described above with the carrier which constitutes one or more necessary compositions ingredients. general, the In

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prepared by uniformly and intimately admixing the immunogenic agents of the invention with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation.

The above compositions may be administered in a manner compatible with the dosage formulation, and in such amount as is immunogenically-effective to protect patients from N. meningitidis infection. dose administered to a patient, in the context of the present invention, should be sufficient to effect a beneficial response in a patient over time such as a reduction in the level of N. meningitidis, or to inhibit infection by N. meningitidis. The quantity of the immunogenic agent(s) to be administered may depend on the subject to be treated inclusive of the age, sex, weight and general health condition thereof. In this regard, precise amounts of the immunogenic agent(s) required to be administered will depend on the judgement of the practitioner. In determining the effective amount of the immunogenic agent to be administered in the treatment or prophylaxis against may evaluate the physician N. meningitidis, circulating plasma levels, progression of disease, and the production of anti-N. meningitidis antibodies. any event, suitable dosages of the immunogenic agents of the invention may be readily determined by those of skill in the art. Such dosages may be in the order of nanograms to milligrams of the immunogenic agents of the invention.

The above compositions may be used as therapeutic or prophylactic vaccines. Accordingly, the invention extends to the production of vaccines containing as actives one or more of the immunogenic

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agents of the invention. Any suitable procedure is contemplated for producing such vaccines. Exemplary procedures include, for example, those described in NEW GENERATION VACCINES (1997, Levine et al., Marcel Dekker, Inc. New York, Basel Hong Kong) which is incorporated herein by reference.

An immunogenic agent according to the invention can be mixed, conjugated or fused with other antigens, including B or T cell epitopes of other antigens. In addition, it can be conjugated to a carrier as described below.

When an haptenic peptide of the invention is a peptide which reacts with cognate (i.e., used but cannot itself elicit immune an antibodies, response), it can be conjugated with an immunogenic Useful carriers are well known in the art and include for example: thyroglobulin; albumins such as human serum albumin; toxins, toxoids or any mutant the toxin from crossreactive material (CRM) of tetanus, diptheria, pertussis, Pseudomonas, E. polyamino and Streprococcus; Staphylococcus, influenza; poly(lysine:glutamic acid); Rotavirus VP6, Parvovirus VP1 and VP2; hepatitis B virus core protein; hepatitis B virus recombinant Alternatively, a fragment or vaccine and the like. epitope of a carrier protein or other immnogenic protein may be used. For example, a haptenic peptide of the invention can be coupled to a T cell epitope of a bacterial toxin, toxoid or CRM. In this regard, reference may be made to U.S. Patent No 5,785,973 which is incorporated herein by reference.

In addition, a polypeptide, fragment, variant or derivative of the invention may act as a carrier

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protein in vaccine compositions directed against Neisseria, or against other bacteria or viruses.

The immunogenic agents of the invention may be administered as multivalent subunit vaccines in combination with antigens of N. meningitidis, or organisms inclusive of other of antigens pathogenic bacteria H. influenzae, M. catarrhalis, N. E. coli, S. pneumoniae etc. gonorrhoeae, Alternatively additionally, they may or with oligosaccharide or administered concert in polysaccharide components of N. meningitidis.

The vaccines can also contain a physiologically-acceptable diluent or excipient such as water, phosphate buffered saline and saline.

The vaccines and immunogenic compositions may 15 include an adjuvant as is well known in the art. Suitable adjuvants include, but are not limited to: surface active substances such as hexadecylamine, acid octadecyl / amino octadecylamine, lysolecithin, dimethyldioctadecylammonium bromide, N, 20 N-dicoctadecyl-N', N'bis(2-hydroxyethylmethoxyhexadecylglycerol, and propanediamine), polyamines such as polyols; pluronic dextransulfate, poly IC carbopol; peptides such as muramyl dipeptide and derivatives, dimethylglycine, 25 tuftsin; oil emulsions; and mineral gels such as aluminum phosphate, aluminum hydroxide or lymphokines, QuilA and immune stimulating complexes (ISCOMS).

The immunogenic agents of the invention may be expressed by attenuated viral hosts. By "attenuated viral hosts" is meant viral vectors which are either naturally, or have been rendered, substantially avirulent. A virus may be rendered

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substantially avirulent by any suitable physical chemical means (e.g., heat treatment) or formaldehyde treatment). By "substantially avirulent" is meant a virus whose infectivity has been destroyed. Ideally, the infectivity of the virus is destroyed without affecting the proteins which carry the immunogenicity of the virus. From the foregoing, it will be appreciated that attenuated viral hosts may comprise live viruses or inactivated viruses.

Attenuated viral hosts which may be useful in a vaccine according to the invention may comprise viral vectors inclusive of adenovirus, cytomegalovirus and preferably pox viruses such as vaccinia (see for example Paoletti and Panicali, U.S. Patent 4,603,112 which is incorporated herein by reference) and attenuated Salmonella strains (see for example Stocker, U.S. Patent No. 4,550,081 which is herein Live vaccines incorporated by reference). particularly advantageous because they lead to a prolonged stimulus which can confer substantially long-lasting immunity.

Multivalent vaccines can be prepared from one or more microorganisms that express different epitopes of N. meningitidis (e.g., other surface proteins or epitopes of N. meningitidis). In addition, epitopes of other pathogenic microorganisms can be incorporated into the vaccine.

In a preferred embodiment, this will involve the construction of a recombinant vaccinia virus to express a nucleic acid sequence according to the invention. Upon introduction into a host, the recombinant vaccinia virus expresses the immunogenic agent, and thereby elicits a host CTL response. For example, reference may be made to U.S. Patent No

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4,722,848, incorporated herein by reference, which describes vaccinia vectors and methods useful in immunization protocols.

A wide variety of other vectors useful for therapeutic administration or immunization with the immunogenic agents of the invention will be apparent to those skilled in the art from the present disclosure.

In a further embodiment, the nucleotide sequence may be used as a vaccine in the form of a "naked DNA" vaccine as is known in the art. For example, an expression vector of the invention may be introduced into a mammal, where it causes production of a polypeptide in vivo, against which the host mounts an immune response as for example described in Barry, M. et al., (1995, Nature, 377:632-635) which is hereby incorporated herein by reference.

### Detection kits

The present invention also provides kits for 20 the detection of N. meningitidis in a biological These will contain one or more particular agents described above depending upon the nature of the test method employed. In this regard, the kits may include one or more of a polypeptide, fragment, 25 variant, derivative, antibody, antibody fragment or nucleic acid according to the invention. The kits may also optionally include appropriate reagents detection of labels, positive and negative controls, washing solutions, dilution buffers and the like. 30 a nucleic acid-based detection example, include (i) a nucleic acid according to the invention (which may be used as a positive control), (ii) an oligonucleotide primer according to the invention, and

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optionally a DNA polymerase, DNA ligase etc depending on the nucleic acid amplification technique employed.

# Preparation of immunoreactive fragments

The invention also extends to a method of fragment immunoreactive an identifying polypeptide, variant or derivatives according to the comprises essentially method invention. This generating a fragment of the polypeptide, variant or derivative, administering the fragment to a mammal; and detecting an immune response in the mammal. response will include production of elements which meningitidis and/or said specifically bind N. and/or derivative, variant or polypeptide, protective effect against N. meningitidis infection.

Prior to testing a particular fragment for immunoreactivity in the above method, a variety of predictive methods may be used to deduce whether a particular fragment can be used to obtain an antibody that cross-reacts with the native antigen. predictive methods may be based on amino-terminal or carboxy-terminal sequence as for example described in Chapter 11.14 of Ausubel et al., (1994-1998, supra). Alternatively, these predictive methods may be based on predictions of hydrophilicity as for described by Kyte and Doolittle (1982, J. Mol. Biol. 157:105-132) and Hopp and Woods (1983, Mol. Immunol. incorporated by reference 20:483-489) which are herein, or predictions of secondary structure as for example described by Choo and Fasman (1978, Ann. Rev. Biochem. 47:251-276) which is incorporated herein by reference.

Generally, peptide fragments consisting of 10 to 15 residues provide optimal results. Peptides as

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small as 6 or as large as 20 residues have worked successfully. Such peptide fragments may then be chemically coupled to a carrier molecule such as keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA) as for example described in Sections 11.14 and 11.15 of Ausubel et al., (1994-1998, supra).

The peptides may be used to immunize an animal as for example discussed above. Antibody titers against the native or parent polypeptide from which the peptide was selected may then be determined by, for example, radioimmunoassay or ELISA as for instance described in Sections 11.16 and 114 of Ausubel et al., (1994-1998, supra).

Antibodies may then be purified from a suitable biological fluid of the animal by ammonium sulfate fractionation or by chromatography as is well known in the art. Exemplary protocols for antibody purification is given in Sections 10.11 and 11.13 of Ausubel et al., (1994-1998, supra).

Immunoreactivity of the antibody against the native or parent polypeptide may be determined by any suitable procedure such as, for example, western blot.

### Functional blockers

The polypeptides according to SEQ ID NOS 2, 5, 7, 9, 25 11, 13, 15, 17, 19 and 21 are believed to have adhesin They in fact have some similarity to adhesins of Haemophilus influenzae which are surface Specifically they are approximately 67% antigens. influenzae to the Hia protein of H.30 homologous (Barenkamp, S. and St. Geme III, J. 1996 Molecular Microbiology 19: 1215-1233), and 74% homologous to the Hsf protein of H. influenzae (St. Geme III, J. et al, 1996, Journal of Bacteriology 178: 6281-6287; and U.S.

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Patent No 5,646,259). For these comparisons, a gap weight of 3, and length weight of 0.01 was used using the GAP program (Deveraux, 1984, supra). Aligned sequences of these proteins are illustrated in FIG. 6. Thus, interruption of the function of these polypeptides would be of significant therapeutic benefit since they would prevent N. meningitidis bacteria from adhering to and invading cells. Interruption of the function may be effected in several ways.

For example, moieties such as chemical reagents or polypeptides which block receptors on the cell surface which interact with a polypeptides according to SEQ ID NOS 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21 may be administered. These compete with the infective organism for receptor sites. Such moieties may comprise for example polypeptides of the invention, in particular fragments, or functional equivalents of these as well as mimetics.

The term "mimetics" is used herein to refer to chemicals which are designed to resemble particular functional regions of the proteins or peptides. Antiidiotypic antibodies raised against the abovedescribed antibodies which block the binding of the to a cell surface may also be used. bacteria interact with the Alternatively, moieties which receptor binding sites in the polypeptides according to SEQ ID NO 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21 may effectively prevent infection of a cell by Such moieties may comprise blocking meningitidis. antibodies, peptides or other chemical reagents.

All such moieties, pharmaceutical compositions in which they are combined with pharmaceutically acceptable carriers and methods of

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treating patients suffering from N. meningitidis infection by administration of such moieties or compositions form a further aspect of the invention.

The polypeptides of the invention may be used in the screening of compounds for their use in the For example, polypeptides of the above methods. invention may be combined with a label and exposed to a cell culture in the presence of a reagent under The ability of reagent to inhibit the binding of the labeled polypeptide to the cell surface can In such a screen, the labeled then be observed. polypeptides may be used directly on an organism such as E. coli. Alternatively, N. meningitidis itself may be engineered to express a modified and detectable form of the polypeptide. The use of engineered N. meningitidis strains in this method is preferred as it is more likely that the tertiary structure of the protein will resemble more closely that expressed in wild-type bacteria.

In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

### EXAMPLE 1

Molecular cloning and subcloning and hiaNm mutant construction.

The hiaNm gene was initially isolated by PCR amplification using standard methods. Briefly, due to our previous work on homologues of the AIDA-I protein of E. coli (Jennings, M. et al, 1995, Microbial Pathogenesis, 19: 391-407, Peak, I. et al, Microbial Pathogenesis, in press) we performed a homology

search, identifying а sequence of interest in preliminary data from the project to sequence the genome of MC58¢3 (The Institute for Genomic Research, (ftp://ftp.tigr.org/pub/data/n meningitidis/) amplified the region of homology by PCR (polymerase 5 chain reaction) using oligonucleotides A3A (5' -TTTGCAACGGTTCAGGCA-3', SEQ ID NO 28) and A3B (5' -TATTCAGCAGCGTATCGG-3', SEQ ID NO 29). The resulting 449 base pairs (bp) product was cloned into pT7Blue, to create plasmid pNMAIDA3. To clone the full length 10 gene, further oligonucleotides were designed and used in an inverse PCR reaction. These oligonucleotides were A3C (SEQ ID NO 30) and A3D (SEQ ID NO 31) correspond to the complementary sequence of A3A (SEQ ID NO 28) and A3B (SEQ ID NO 31) respectively. 15 template for this reaction was chromosomal DNA of MC58 which had been restriction digested with EagI and then The resulting 3kbp PCR product was self ligated. cloned into the vector pCRII (Invitrogen), producing This was digested with EagI and plasmid piEagA3. 20 EcoRI and the resulting fragments of 1.4kbp and 1.6kbp into were cloned cloned DNA containing pBluescriptSKII, M13minus (Stratagene), resulting in piEagA3.8 and piEagA3.9. Plasmid pHiaNm was generated by PCR amplifying hiaNm and sequence 5' and 3' 25 (5'oligonucleotide primers HiaNm: P using TTAGATTCCACGTCCCAGATT-3', SEQ ID NO 22) and HiaNm:M NO 23), (5'-CTTCCCTTCAAACCTTCC-3', SEO ID corresponding to nucleotide position (ntp) 113-133 and 2102-2085 respectively of SEQ ID NO 1, and cloning the 30 product into pT7Blue. Plasmid pHiaNmAKan was created by insertion of a kanamycin resistance cassette into the unique BglII site of pHiaNm corresponding to ntp 680 of SEQ ID No 1. The kanamycin resistance cassette

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was excised from pUC4Kan (Pharmacia) with BamHI. pHiaNmΔKan was transformed into N. meningitidis strain MC58 by incubating bacteria with plasmid DNA for 3 hours on Brain Heart Infusion agar (Acumedia Manufacturer's Inc) supplemented with 10% heated horse blood ("BHI plates") at 37°C in 5% CO<sub>2</sub>. A single colony was picked onto fresh selective media, grown, and used for further studies. This mutant strain is designated MC58ΔHiaNm. Disruption of the hiaNm gene in this strain was confirmed by Southern blot using a probe corresponding to ntp 276-2054 of SEQ ID NO 1.

### EXAMPLE 2

### Nucleotide sequence analysis

Nucleotide sequence analysis was performed using the PRISM Dye terminator sequencing Kit with AmpliTaq DNA polymerase FS or BigDye terminator sequencing kit as suggested by the manufacturer's instructions (Perkin Elmer), in conjunction with a model 373a automated sequencer (Applied Biosystems). each strain, hiaNm was amplified independent PCR reactions using primers HiaNm5'A2: 5'-CCAAACCCCGATTTAACC-3' (SEQ ID NO 26) and HiaNm3'A: 5'-AATCGCCACCCTTCCCTTC-3' (SEQ ID NO 27), as indicated on FIG. 1, and corresponding to ntp 230-247 and 2114-2097 of SEQ ID No 1, and the resulting products purified This was used as template for direct and pooled. sequencing on both strands. Data were analysed using the GCG programs (Deveraux et al. (1984) Nucleic Acids Research 12, 387-395) and AssemblyLIGN (Oxford Molecular). Several oligonucleotides were generated as necessary to complete sequences. Sequences of hiaNm of 10 strains are shown in SEQ ID NOS 1, 3, 4,

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6, 8, 10, 12, 14, 16, 18, and 20, and the deduced amino acid sequences of those genes are shown in SEQ ID NO 2, 5, 7, 9, 11, 13, 15, 17, 19 and 21.

Comparison of hiaNm from these strains indicated that they share 90-99% identity with hiaNm In addition, hiaNm of MC58 is 62% and 68% of MC58. homologous to hia and hsf of Haemophilus influenzae However, in the strains examined, hiaNm is 1770-1800 bp long. This is markedly different from the hia and hsf which are 3294 and 7059 bp long respectively. The predicted polypeptide of hiaNm, HiaNm, also exhibits several other bacterial proteins, to homology including AIDA-I, the adhesin involved in diffuse adherence of the diarrhoeagenic Escherichia coli strain 2787 (0126:H27), HMW1, another Haemophilus adhesin, UspA1, a high molecular weight protein of Moraxella catarrthalis, and SepA involved in tissue invasion of Shigella flexneri (Benz, I. Schmidt, M.A., 1992, Molecular Microbiology 6:1539-1546, Barenkamp, S.J. and Leininger, E. 1992, Infection et.al 1302-1313, Aebi, C. and Immunity 60: 1997, Infection and Immunity 65: 4367-4377, Benjelloun-Touimi, Z et al 1995, Molecular Microbiology 17:123-135). Homology to these (and several other proteins) occurs over the first fifty amino acids of HiaNm. Analysis of this sequence reveals the presence of a predicted signal sequence, with cleavage sites at amino acid 50 in all HiaNm sequences examined. long signal sequences are common to proteins located in the outer membrane of Gram-negative bacteria (Henderson, I et al, 1998, Trends in Microbiology 6: The proteins mentioned above to which the 370-8). first fifty amino acids of HiaNm is homologous are all of the "autotransporter" outer-membrane members

protein family (Henderson, I, *supra*). This strongly suggests that HiaNm is located in the outer membrane of *N. meningitidis*.

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#### EXAMPLE 3

### Southern blot analysis

Southern blot analysis was performed using standard techniques (Sambrook et al., supra, Ausubel Briefly, genomic DNA was prepared et al., supra). meningitidis of several from 70 strains of N. serogroups, restriction digested ar.d separated gel prior to electrophoretically on an agarose capillary transfer to a nylon membrane. membranes were hybridized with a labeled probe. probe used corresponded to ntp 276-2054 of SEQ ID NO 1, encompassing the entire open reading frame of hiaNm of strain MC58. This was labeled with (dioxygenin) according to manufacturer's instructions (Boehringer Mannheim). Stringent washes performed (two washes of 5 minutes at 22°C in 2 x SSC/0.1% SDS followed by two washes of 30 minutes, 68°C, 0.2 x SSC/0.1% SDS). Hybridization was detected colorimetrically using nitro-blue-tetrazolium/ bromochloryl-indolyl-phosphate (NBT/BCIP) as recommended by the manufacturer. Signals were detected in all strains examined. (FIG. 2 for example). In addition to the prototypic strain MC58, the following strains were investigated:

## TABLE 3

actain name	BOULLE		arram num		
PMC 3 (J1079)	2*	A	NGF26	1	В.

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				•	
PMC17 (K874)	2	Α	NGG40	1	В
PMC 20 ((H79)	2	A	H15	1.	В
PMC23 (K750)	2	A	SWZ107	1 ·	В
PMC 12 (K852)	2	В	528	1 ·	В.
PMC 13 (K859)	2	В.	2970	1	В ;,:
PMC 16 (K873)	2	В	1000	1	В
PMC 24 (K782)	2	В	MPJB28	3 <sup>c</sup>	В
PMC 25 (K791)	2 ,	В.	MPJB56	3	В
PMC 27 (K816)	2	В	мрјв88	3	В
PMC 28 (K837)	2	В .	МРЈВ157	3	В
BZ10	1 <sup>B</sup>	В .	мрјВ328	3	В
BZ47	1	В	мрјв627	3	В
BZ83	1	В	MPJB820	3	В
BZ133	1.	P	MPJB945	3	В
BZ147	1	В	PMC 8 (K157)	2	C Salar
BZ163	1	В	PMC 9 (K497)	2	c
BZ169	1	В	PMC 11 (K848)	2	С
BZ198	1	В	PMC 14 (K860)	2	С
BZ232	1	В	PMC 18 (K879)	2	С
NG3/88	1	В	PMC 21 (K656)	2	С
NG4/88	1	В	PMC 29 (K841)	2	С
NG6/88	1	В	MPJC05	3	С
EG327	1	В	MPJC14	3.	C
EG329	1	В	мрјс154	3	С
DK353	1.	В	MPJC302	3	C
179/82	1	В	мрјс379	3	C N
66/84	1:	В	PMC19	2	W
DK24	1	В	MPJW025	3	W
<b>NGH36</b>	1	В	PMC 1 (J603)	2	х
н38	1	В	PMC 6 (K131)	2	x
H41	1	В	PMC 10 (K526)	2	Y .
NGE28	1	В	PMC 22 (K685)	2	Y
NGE30	1	В :	PMC 26 (K810)	2	<b>Y</b> ,
NGP20	1	В	PMC 2 ((J1049)	2	z
<u> </u>			<u> </u>		

A World Health Organization Collaborating Centre for Reference and Research on Meningococci, Oslo, Norway B Public Health Laboratory Service Meningococcal

<sup>5</sup> Reference Laboratory, Manchester, UK

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<sup>c</sup> Brisbane Hospitals, now in strain collection of M.P. Jennings, Department of Microbiology, University of Queensland, Brisbane, Australia.

EXAMPLE 4

# Expression and partial purification of MBP-HiaNm

constructed A plasmid vector was permitted the expression of a protein consisting of a fusion of Maltose Binding Protein and HiaNm (MBPplasmid pHiaMBP was generated by HiaNm). The amplifying hiaNm from MC58 using primers Hianm-MBPA 5'-GGTCGCGGATCCATGAACAAAATATACCGCAT-3' (SEQ ID NO 24) and HiaNm-MBPB 5'-TCACCCAAGCTTAAGCCCTTACCACTGATAAC-3' (SEQ ID NO 25). These primers encompass the start and stop codons of hiaNm of N. meningitidis strain MC58 and engineered restriction sites for ease of cloning. of positions maps and restriction Plasmid oligonucleotides are shown in FIG. 1. The resultant PCR product was ligated into BamHI/HindIII restriction digested plasmid pMALC2 (New England Biolabs), and the resultant plasmid, pHiaMBP (See FIG. 1) reintroduced coli This E. E . coli strain DH5 $\alpha$ . containing pHiaMBP was induced to express the HiaNm-MBP fusion protein under conditions recommended by the Cell extracts manufacturer (New England Biolabs). from cultures containing pHiAMBP were separated by 10% fusion protein was partially and the purified by elution using the Mini-Gel Electro-eluter to manufacturer's instructions. 30 according (BioRad) Fractions containing the HiaNm-MBP fusion protein were detected by Western blot using rabbit anti-MBP sera (New England Biolabs). The purity of the HiaNm-MBP

fusion protein was determined by SDS-PAGE followed by Coomassie staining, and the amount of recovered protein estimated by BCA assay (Sigma) or absorbance at a wavelength of 280nm.

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### EXAMPLE 5

### Generation of polyclonal sera

partially purified HiaNm-MBP fusion protein obtained in Example 4 was used to generate polyclonal sera in rabbits. Samples of eluted HiaNmMBP fusion protein were dialyzed against sterile phosphate buffered saline pH 7.4, (PBS) (Sigma). This was then (MPL+TDM+CWS, Sigma), adjuvant mixed with concentration of 50-150µg/mL and inoculated at two weekly intervals into two New Zealand White rabbits. rabbits. Serum from these was taken Blood extracted by clotting at room temperature for one hour 4°C before at followed by overnight incubation centrifugation at 4000 x rpm at 4°C. The supernatant was removed and re-centrifuged. Serum was stored in Sera obtained were used aliquots at -80°C. bactericidal assays and Western blots (see below).

To test the specificity of the sera obtained, Briefly, undertaken. Western blot analysis was MC58 N. meningitidis strains proteins of  $MC58\Delta Hianm$  were separated electrophoretically on SDS-PAGE before electrophoretic transfer to nitrocellulose membrane using a Semi-Dry Blotter (BioRad). These sera and incubated sequentially with then IqG anti-Rabbit conjugated alkaline-phosphatase (Sigma) before colorimetric detection with NBT/BCIP (Sigma). These experiments demonstrated that antibodies were elicited by the HiaNm-MBP fusion protein which

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were specific for, and detected a band in, MC58 but in MC58∆HiaNm (see FIG. 4). The predicted molecular weight of the deduced polypeptide of HiaNm The band detected by the sera migrates is 62.3 kDa. at an apparent MW in excess of 150 kDa. three of the homologous "autotransporter" proteins reported in the literature also display such anomalous migration: the high molecular weight outer membrane proteins UspA1 and UspA2 of Moraxella catarrhalis have predicted molecular weights of 62.5 kDa and 88.3 kDa respectively but migrate at 85 kDa and 120 kDa, respectively and as the UspA complex at between 350 kDa and 720 kDa (Aebi, C. et al., 1997, Infection and Immunity, 65: 4367-4377, Klingman, K.L. and Murphy, T.F., 1994, Infection and Immunity, 62: 1150-1155). Similarly, Hia of Haemophilus influenzae has predicted molecular weight of 116 kDa but when expressed in a phage, Hia migrates at greater than 200 kDa (Barenkamp, S. and St. Geme III, J. 1996 Molecular Microbiology 19: 1215-1233).

In order to confirm that HiaNm is associated with the outer membrane of N. meningitidis, outer membrane complexes (omc) were prepared, essentially as previously described (van der Ley, P. et al, 1991, Immunity, 59:2963-71). Infection and bacteria were grown overnight on Brain Heart Infusion agar (Acumedia Manufacturer's Inc) supplemented with 10% heated horse blood BHI plates, resuspended in 10 mM Tris pH 8.0 and heat killed, before sonication to disrupt the membrane. Cellular debris were removed by centrifugation at 10,000 X g (rcf, centrifugal force), and the supernatant recentrifuged at 50,000 x q. This pellet was resuspended in 1% sarkosyl/10 mM Tris pH8.4 and centrifuged at 10,000 x

The supernatant was centrifuged at 75,000 x g and the pellet resuspended in Tris pH 8.4, before quantification spectrophotometrically at a wavelength of 280nm. An aliquot of the sarkosyl-insoluble fraction, which contains outer membrane proteins, (50 $\mu$ l of  $A_{280}$ =3.75) was subjected to SDS-PAGE and Western blotted as described above. The results, shown in FIG. 4 demonstrate that reactivity with the anti-HiaNmMBP antisera is observed with wild type MC58, but not with MC58ΔHiaNm, in which hiaNm has The increase in reactivity with the inactivated. anti-HiaMBP sera observed between whole cell samples, and the omc samples containing the same amount of total protein, in MC58 cultures is consistent with the membrane association of HiaNm.

#### EXAMPLE 6

#### Bactericidal assay

To determine whether the anti-HiaMBP antisera contained bactericidal antibodies specific for HiaNm, bactericidal assays were performed with wild type MC58 and MC58AHiaNm. This assay was performed by a modification of the method described by Hoogerhout et. al. (1995, Infection and Immunity, 63: 3473-3478). Briefly, MC58 and MC58 AHiaNm were grown overnight on BHI plates at 37°C in 5% CO2. Bacteria from this overnight culture were subcultured under the conditions for 4-6 hours before suspension in 1 mL PBS. Numbers of bacteria were estimated by lysis of a sample in 0.2N NaOH/1% SDS and absorbance at a wavelength of 260 nm, where  $A_{260}=1 = 10^9$  cfu/mL. bacterial suspension was adjusted to approximately 105 cfu/mL in PBS. Rabbit sera to be tested was heat

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inactivated at 56°C for 45 minutes. Serum from four week old, New Zealand White rabbits was pooled and used source of complement (Central Breeding House, University of Queensland). was carried out in sterile polystyrene flat-bottomed 96 well microtitre plate. The total volume in each well was 24 μL: 12 μL of twofold serially diluted serum in PBS and 6 µL of bacterial suspension (containing between 300-900 bacteria). Sera and bacteria were incubated at room temperature for 10 minutes before addition of 6 µL of 80% complement in PBS (final Controls were concentration 20% vol/vol). bacteria and complement, b) PBS, bacteria and serum. After addition of all components and mixing, a 7  $\mu L$ aliquot from each control well was spread on a BHI plate. The microtitre plate was then incubated at 37°C in 5% CO2 for 60 minutes. After this incubation, a 7 μL aliquot from each well was spread on BHI plates. All BHI plates were then incubated for 14-18 hours at 37°C in 5% CO2, and bacterial colonies counted. Serum bactericidal killing is reported highest as the reciprocal dilution at which at least 90% of bacteria were killed. Serum used was from the same rabbit and test bleed as used for Western blot the experiments as reported in Example 5 above. experiments consistently demonstrated reduced titers (approximately 3 fold, Table 4) of killing against MC58ΔHiaNm in comparison to the wild type strain, the anti-HiaMBP antisera MC58, indicating that contained bactericidal antibodies specific for HiaNm.

TABLE 4



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MC58	12 (+/- 4.6)		
MC58∆HiaNm	3.5 (+/- 1)		

<sup>&</sup>lt;sup>a</sup> Mean of four independent experiments

### DISCUSSION

Repetitive DNA has been associated with virulence determinants in some pathogenic bacteria. Southern blots using such a repetitive DNA motif revealed the presence of at least three loci which contained this motif in N. meningitidis strain MC58 (Peak, I. et al., 1996, FEMS Microbiology Letters, These genes were cloned and sequence 137:109-114). analysis of two of these repeat associated loci (nmrep2 and nmrep3) revealed open reading frames of approximately 670 amino acids (Jennings, M. et al, 1995, Microbial Pathogenesis, 19: 391-407, Peak, I. et 15 Pathogenesis, in press). Microbial al, exhibited homology to each other and homology to the carboxyl-terminal of the adhesin AIDA-I of E. coli. The carboxyl-AIDA-I is 1286 amino acids long. terminal region constitutes a putative outer membrane 20 transport domain and the amino-terminal domain of the mature protein constitutes the adhesin domain. amino-terminal domain crosses the membrane through the putative transport domain and is designated 25 passenger domain.

As Nmep2 and Nmep3 share sequence homology with the transporter domain of AIDA-I, thought to form membrane pores. Nmrep2 and Nmrep3 are approximately half the size of AIDA-I, homologous to the membrane spanning domain of AIDA-I. We hypothesized that there existed in N. meningitidis

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a locus with homology to the amino-terminal domain of AIDA-I. We searched for such a homologue in the data from the project to sequence N. meningitidis strain MC58¢3 (TIGR, supra) and found one region with homology to a gene designated AIDA-I in Haemophilus influenzae strain Rd (HI1732) because of its homology to AIDA-I of E. coli, (Fleischmann et. al., 1995 Science 269:496-512,). In view of the homologies noted above, the applicants decided to investigate further.

The gene was initially isolated by PCR amplification of the DNA corresponding to the 471 base pair fragment, named gnmaa84r, from N. meningitidis Further PCR MC58 3 and the sequence was confirmed. experiments enabled larger fragments to be amplified. These were cloned and sequence analysis undertaken as shown in FIG 1. The gene exhibited homology to the amino-terminal region of AIDA-I of E. coli and we designated it aida3, as it represented the third AIDA-I homologue in N. meningitidis (with nmrep2 and Since then, the discovery of two further hia and hsf from H. influenzae has been published (Barenkamp, S. and St. Geme III, J. 1996 Molecular Microbiology 19: 1215-1233, St. Geme III, J. et al, 1996, Journal of Bacteriology 178: 6281-6287), to which aida3 is more similar. We have therefore redesignated this gene hiaNm. (HI1732, the H. influenzae gene first identified as an homologue of AIDA-I has also been re-designated hia in light of the reports of Barenkamp and St. Geme III).

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Throughout the specification the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. It will therefore

be appreciated by those of skill in the art that, in light of the instant disclosure, various modifications and changes can be made in the particular embodiments exemplified without departing from the scope of the present invention. All such modifications and changes are intended to be included within the scope of the appendant claims

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### CLAIMS

- 1. An isolated polypeptide or fragment thereof, or variant or derivative of these, said polypeptide selected from the group consisting of:
- 5 (a) a polypeptide according to SEQ ID NO 2;
  - (b) a polypeptide according to SEQ ID NO 5;
  - (c) a polypeptide according to SEQ ID NO 7;
  - (d) a polypeptide according to SEQ ID NO 9;
  - (e) a polypeptide according to SEQ ID NO 11;
  - (f) a polypeptide according to SEQ ID NO 13;
  - (g) a polypeptide according to SEQ ID NO 15;
  - (h) a polypeptide according to SEQ ID NO 17;
  - (i) a polypeptide according to SEQ ID NO 19; and
- 15 (j) a polypeptide according to SEQ ID NO 21.
  - 2. A polypeptide, fragment, variant or derivative according to claim 1, displaying immunological activity against one or more members selected from the group consisting of:-
    - (i) N. meningitidis;
    - (ii) said polypeptide;
    - (iii) said fragment;
    - (iv) said variant; and
- 25 (v) said derivative;
  - 3. A polypeptide, fragment, variant or derivative according to claim 1, displaying immunological activity against N. meningitidis.
  - 4. An isolated nucleic acid sequence encoding a polypeptide or fragment thereof, or variant or derivative of these, said polypeptide selected from the group consisting of:

	(a) a polypeptide according to SEQ ID NO 2;
	(b) a polypeptide according to SEQ ID NO 5;
	(c) a polypeptide according to SEQ ID NO 7;
	(d) a polypeptide according to SEQ ID NO 9;
5	(e) a polypeptide according to SEQ ID NO 11;
	(f) a polypeptide according to SEQ ID NO 13;
	(g) a polypeptide according to SEQ ID NO 15;
	(h) a polypeptide according to SEQ ID NO 17;
	<ul><li>(i) a polypeptide according to SEQ ID NO 19;</li></ul>
10	and
	(j) a polypeptide according to SEQ ID NO 21.
	5. An isolated nucleic acid sequence according
	to claim 4, encoding a product displaying
15	immunological activity against one or more members
•	selected from the group consisting of:-
	(i) N. meningitidis;
	<pre>(ii) said polypeptide;</pre>
	(iii) said fragment;
20	(iv) said variant; and
	(v) said derivative.
	a de la companya according
	6. An isolated nucleic acid sequence according to claim 4, encoding a product displaying
	to craim 1, chocally
25	immunological activity against N. meningitidis.
	7. An isolated nucleic acid sequence selected
	from the group consisting of:  (1) the nucleotide sequence of SEQ ID NO 1;
20	(1) the nucleotide sequence of SEQ ID NO 1; (2) the nucleotide sequence of SEQ ID NO 3;
30	(3) the nucleotide sequence of SEQ ID NO 4;
	(4) the nucleotide sequence of SEQ ID NO 6;
	(5) the nucleotide sequence of SEQ ID NO 8;
-1	(6) the nucleotide sequence of SEQ ID NO 10;
. 25	(7) the nucleotide sequence of SEQ ID NO 12;
35	(/) the morocata bolasses a

- (8) the nucleotide sequence of SEQ ID NO 14;
- (9) the nucleotide sequence of SEQ ID NO 16;
- (10) the nucleotide sequence of SEQ ID NO 18;
- (11) the nucleotide sequence of SEQ ID NO 20;
- (12) a nucleotide sequence fragment of any one of SEQ ID NOS 1, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 20; and
- (13) a nucleotide sequence homologue of any of the foregoing sequences

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8. A nucleic acid sequence according to claim 7, encoding a product displaying immunological activity against one or more members selected from the group consisting of:-

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- (i) N. meningitidis;
- (ii) said polypeptide;
- (iii) said fragment;
- (iv) said variant; and
- (v) said derivative.

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- 9. A nucleic acid sequence according to claim 7, encoding a product displaying immunological activity against N. meningitidis.
- 25 10. The nucleic acid sequence of claim 7, wherein said homologue is obtained from the genus Neisseria.
  - 11. The nucleic acid sequence of claim 5 or claim 7, wherein said homologue is obtained from a strain of N. meningitidis.
  - 12. A method of obtaining a nucleotide sequence homologue comprising the steps of:-
    - (i) obtaining a nucleic acid extract from a suitable host;

Substitute **Sheet** (Rule 26) **RO/AU** 

- (ii) creating primers which are optionally degenerate wherein each comprises a portion of a nucleic acid sequence according to claim 5 or claim 7; and (iii) using said primers to amplify, via a
  - (iii) using said primers to amplify, via a nucleic acid amplification technique, one or more amplification products from said nucleic acid extract.
- 10 13. The method of claim 12, wherein said nucleic acid extract is obtained from the genus Neisseria.
- 14. The method of claim 12, wherein said nucleic acid extract is obtained from a strain of N.15 meningitidis.
  - 15. The method of claim 12, wherein said primers are selected from the group consisting of SEQ ID NOS 22, 23, 24, 25, 26, 27, 28, 29, 30, and 31.
- 20
  16. The method of claim 12, wherein the nucleic acid amplification technique is PCR.
- 17. An expression vector comprising a nucleic acid sequence according to claim 4 or claim 7, wherein said sequence is operably linked to transcriptional and translational regulatory nucleic acid.
- 18. A host cell transfected or transformed with an expression vector comprising a nucleic acid sequence according to claim 4 or claim 7, wherein said sequence is operably linked to transcriptional and translational regulatory nucleic acid.

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- 19. A method of producing a recombinant polypeptide comprising the steps of:
  - (A) culturing a host cell according to claim

    18 such that said recombinant
    polypeptide is expressed from said
    nucleic acid; and
    - (B) isolating said recombinant polypeptide.
- 20. An antibody or antibody fragment which binds

  10 to one or more members selected from the group

  consisting of:-
  - 🚲 (1) N. meningitidis;
    - (2) a polypeptide according to claim 1;
  - (3) a fragment of said polypeptide;
  - (4) a variant of said polypeptide or said fragment; and
    - (5) a derivative of said polypeptide or said fragment.
- 20 21. The antibody of claim 20, wherein said antibody or antibody fragment binds N. meningitidis.
  - 22. A method of detecting N. meningitidis in a biological sample suspected of containing same, said method comprising the steps of:-
    - (A) isolating the biological sample from a patient;
    - (B) mixing the antibody or antibody fragment of claim 20 or claim 21 with the biological sample to form a mixture; and
    - (C) detecting specifically bound antibody or bound fragment in the mixture which indicates the presence of N. meningitidis.

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- 23. A method of detecting *N. meningitidis* bacteria in a biological sample suspected of containing said bacteria, said method comprising the steps of:-
  - (I) isolating the biological sample from a patient;
  - '(II) detecting a nucleic acid sequence according to claim 4 or claim 7 in said sample which indicates the presence of said bacteria.
- 24. A method for diagnosing infection of patients by N. meningitidis, said method comprising the steps of:-
  - (1) contacting a biological sample from a patient with a polypeptide, fragment, variant or derivative according to claim 1; and
  - (2) determining the presence or absence of a complex between said polypeptide, fragment, variant or derivative and N. meningitidis-specific antibodies in said sample, wherein the presence of said complex is indicative of said infection.
  - 25. Use of the polypeptide, fragment, variant or derivative according to claim 1 for the manufacture of a kit for the detection or diagnosis of N. meningitidis infection in humans.
    - 26. Use of the nucleic acid sequence according to claim 4 or claim 7 for the manufacture of a kit for

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20

10

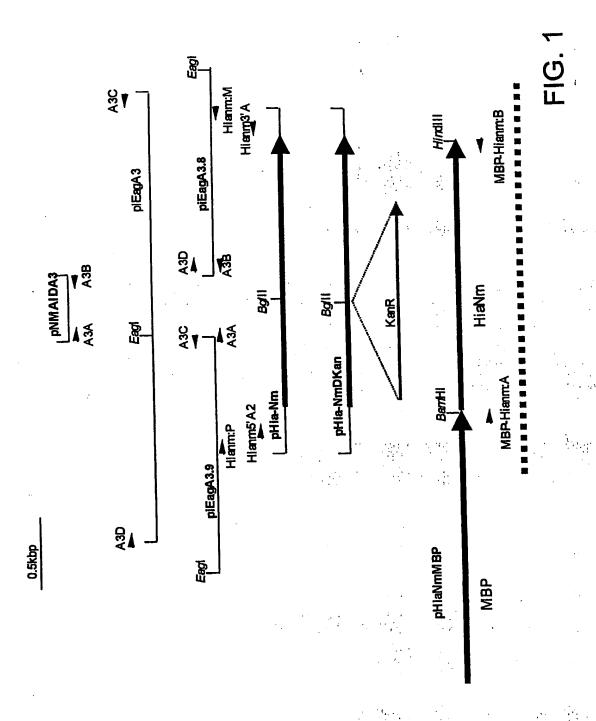
the detection or diagnosis of *N. meningitidis* infection in humans.

- 27. Use of one or more oligonucleotide primers selected from the group consisting of SEQ ID NOS 22, 23, 24, 25, 26, 27, 28, 29, 30 and 31, and optionally a thermostable polymerase, in a kit for the detection or diagnosis of N. meningitidis infection in humans.
- 10 28. Use of the antibody or antibody fragment according to claim 20 or claim 21 for the manufacture of a kit for the detection or diagnosis of N. meningitidis infection in humans.
- 15 29. Use of a pharmaceutically effective amount of a polypeptide, fragment, variant or derivative according to claim 1 for the prevention or treatment of N. meningitidis infection in humans.
- 20 30. Use of a pharmaceutically effective amount of an antibody or antibody fragment according to claim 20 or claim 21 for the prevention or treatment of N. meningitidis infection in humans.
- 25 31. A pharmaceutical composition comprising an isolated polypeptide or fragment thereof, or a variant or derivative of these, according to claim 1.
- 32. The pharmaceutical of claim 31, which is a vaccine.
  - 33. A method of preventing or treating infection of a patient by N. meningitidis, comprising the step

of administrating a pharmaceutically effective amount of a vaccine according to claim 32.

- 34. A method of identifying an immunoreactive fragment of a polypeptide, variant or derivatives according to claim 1, comprising the steps of:-
  - (a) generating a fragment of said polypeptide, variant or derivative;
  - (b) administering said fragment to a mammal; and

detecting an immune response in said mammal which response includes production of elements which specifically bind *N. meningitidis* and/or said polypeptide, variant or derivative, and/or a protective effect against *N. meningitidis* infection.



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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

8 -6 -5 -

FIG. 2A

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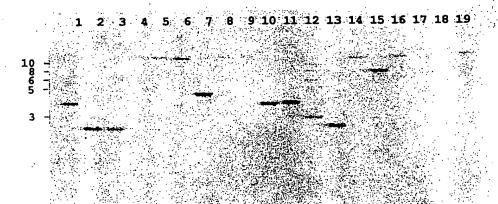


FIG. 2B

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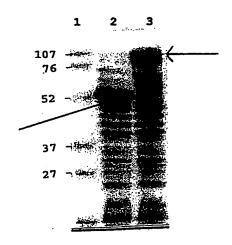


FIG. 3

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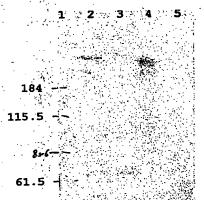


FIG. 4

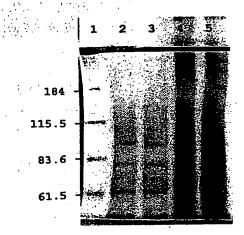


FIG. 5

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FIG. 6 Hsf MNKIFNVIWN VMTQTWVVVS ELTRTHTKRA SATVETAVLA TLLFATVQAN Hia MNKIFNVIWN VVTQTWVVVS ELTRTHTKCA SATVAVAVLA TLLSATVEAN HiaNm MNKIYRIIWN SALNAWVVVS ELTRNHTKRA SATVKTAVLA TLLFATVQAS Hsf ATDEDEELDP VVRTAPVLSF HSDKEGTGEK EVTENSNWGI YFDNKGVLKA Hia GAITLKAGDN LKIKONTDES TNASSFTYSL KKDLTDLTSV ATEKLSFGAN Hsf HiaNm GDKVDITSDA NGLKLAKTGN GNVHLNGLDS TLPDAVTNTG VLSSSSFTPN .....NNTP V..... Hia HiaNm 250 Hsf DVEKTRAATV KDVLNAGWNI KGAKTAGGNV ESVDLVSAYN NVEFITGDKN Hia HiaNm Hsf TLDVVLTAKE NGKTTEVKFT PKTSVIKEKD GKLFTGKENN DTNKVTSNTA HiaNm 350 TDNTDEGNGL VTAKAVIDAV NKAGWRVKTT TANGQNGDFA TVASGTNVTF ...... Hia HiaNm Hsf ESGDGTTASV TKDTNGNGIT VKYDAKVGDG LKFDSDKKIV ADTTALTVTG ...... Hia HiaNm Hsf GKVAEIAKED DKKKLVNAGD LVTALGNLSW KAKAEADTDG ALEGISKDQE Hia HiaNm Hsf VKAGETVTFK AGKNLKVKQD GANFTYSLQD ALTGLTSITL GGTTNGGNDA HiaNm KTVINKDGLT ITPAGNGGTT GTNTISVTKD GIKAGNKAIT NVASGLRAYD Hsf .....LKAYG Hia HiaNm

:;:

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FIG.	6 cont'd			3的第三人称单		
		• .				
	551		·		600	
Hsf	DANFDVLNNS .	ATDLNRHVED	AYKGLLNLNE	KNANKQPLVT	DSTAATVGDL	
	DANFNFTNNS	IADAEKQVQE	AYKGLLNLNE	KNASDKLLVE	DNTAATVGNL	
HiaNm	• • • • • • • • •	•••••	NN	EKPKKKULIL	DPVQRIVAVL	
	C01		M = MM		650	人名英格兰人
**-£	601 RKLGWVVSTK	NCTER SNO				·
Hsf	RKLGWVVSIK	NGTREE: SNO	VKHAD EVIF	EGKGGVOVTS	TSENGKHT	
HiaNm	t ANSDK	EGT. GEKEKV	EENSDWAVYF	NEKGVLT		* * * * * * * * * * * * * * * * * * * *
HIAM		2011021211	-			
	651		14.00 - 14.00 1.00 - 15.00	·	700	1 1. 55
Hsf	VSVAETKADC	GLEKDGDTIK	LKVDNQNTDN	VLTVGNNGTA	VTKGGFETVK	
Hia						
HiaNm						
			•			
_	701		*		750	
Hsf	TGATDADRGK	VTVKDATAND	ADKKVATVKD	VATAINSAAT	FVKTENLTTS	
Hia					••••••	
HiaNm			AND TO SANSO	ลด้องเรียกใหม่ย		
					800	
	751 IDEDNPTONG	WODER WE COM	T TOPEN CENT E	WDDCKNTTF	. ,	
	IDEDNPTONG	KDDALKAGDI	LIFRAGRADA	TTF	ALAKDLGVKT	
Hia	• • • • • • • • •	APF	ITLKAGDNLK	IKONGTNETY	SLKKDLTDLT	22 No. 2 18 18 18 18 18 18 18 18 18 18 18 18 18
HiaNm	. • • • • • • • • • • • • • • • • • • •				$H_{ij}$	
	801				850	
Wef	AKVSTYTTTG	GNTPTGGTTA	TPKVNITSTA	DGLNFAKETA	DASGSKNVYL	
Hia	እጥ <b>ሆ</b> ኖውቸኒጥፐር	GGAAAGATT.	TPKVNVTSTT	DGLKFAKDAA	GANG	
HiaNm		ANGN	KVNITSDT	KGLNFAKETA	GTNG	
			N. A.	•		
•	851				900	
Hsf	KGIATTLTEP	SAGAKSSHVI	) LNVDATKKSN	I AASIEDVLRA	GWNIQGNGNN	
Hia		• • • • • • • •		••••••	••••••	
HiaNm		• • • • • • • • •	• • • • • • • • • • • •		• • • • • • • • •	
		1.			, 950	a a continue
	901	- v-cmppcmcm	η ανευνευτοκατι	Z KGADVKTGAR	TSVIKDHNGK	
		MEIDUSIGI	I IVIVIQUED		• • • • • • • • • •	
Hia						
HiaNm	••••••	ta triger of		339 - 35 34	Harris Harris	造成的原理的
:	951	311113		i i i i i i i i i i i i i i i i i i i	1000	
Hsf	I.FTGKDLKDA	A NNGATVSED	D GKDTGTGLV	T AKTVIDAVNI	K SGWRVTGEGA	
Hia						
HiaNm						
					105	
	1001			10 May 10 Ma	1050	
Hsf	TAETGATAV			A TTATVSKDN	G NINVKYDVN	
Hia					• • • • • • • •	
HiaNm						
					110	o Pariting
	1051		m imceiriein	D CDNG/MINK	k Lvnaeglat	•
Hsf		K KIVADTTTI	T VIGGRVSVE	T GUIDAININ		•
Hia	•	TOTAL				. 1777 - AAA
HiaNn						

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FIG.	6 cont'd
	1101
	LNNLSWTAKA DKYADGESEG ETDQEVKAGD KVTFKAGKNL KVKQSEKDFT
HiaNm	
	1151 1200
Hsf	YSLODTLTGL TSITLGGTAN GRNDTGTVIN KDGLTITLAN GAAAGTDASN
Hia	
HiaNm	
	1201
vr - 6	1201 GNTISVTKDG ISAGNKEITN VKSALKTYKD TQNTADETQD KEFHAAVKNA
Hsf Hia	GNIISVIRDG IDAGMEDITA VEDICATION I PROPERTIES
HiaNm	*********
	1251 1300
Hsf	NEVEFVGKNG ATVSAKTDNN GKHTVTIDVA EAKVGDGLEK DTDGKIKLKV
Hia	
HiaNm	
	1301
Hsf	DNTDGNNLLT VDATKGASVA KGEFNAVTTD ATTAQGTNAN ERGKVVVKGS
HiaNm	
	1351
Hsf	NCATATETOK KKVATVGDVA KAINDAATFV KVENDDSATI DDSPTDDGAN
Hia	
HiaNm	
	1450
Hsf	1401 DALKAGDTLT LKAGKNLKVK RDGKNITFAL ANDLSVKSAT VSDKLSLGTI
Hia	
HiaNm	
	1500
	1451 GNKVNITSDT KGLNFAKDSK TGDDANIHLN GIASTLTDTL LNSGATTNL
Hsf Hia	VHIN GIGSTLTDTL VGSPAIRLD
nia HiaNm	THE RESPONDED TO LONG GRANTING
II. Carvai	155
	1501
Hsf	GNGITDNEKK RAASVKDVLN AGWNVRGVKP ASANNQVENI DFVATYDTV GDQSTHYT RAASIKDVLN AGWNIKGVKA GSTTGQSENV DFVHTYDTV
Hia	GDOSTHYT RAASIKDVLN AGWNIKGVKA GBTTOZDENV DFVRTYDTV NDNVTDDEKK RAASVKDVLN AGWNIKGVKA GTTASDNV DFVRTYDTV
Hianm	
	1551 THE CALL AND
Hsf	THE CONTROL AND THE CYDNICK POTEVIKT CAKET SVIKDINGKL FIGRELIALS
	THE REPORT OF THE PROPERTY OF
HiaNm	FLSADTETTT VIVOSKENGK KTEVKIGVKT SVIKEKDGKL VTGKD.KGE
`	1601
uc <del>f</del>	1601 NNGVTVTETD GKDEGNGLVT AKAVIDAVNK AGWRVKTTGA NGQNDD
	CENTEND DECECTION AKING HONNY TOWATETTO MOSTOCE.
nKe ku	KVD.GANATE DADEGREDVT AKEVIDAVNK AGWRMKTTTA NGQTGQADI

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#### FIG. 6 cont'd 1651 Hsf ATVASGTNVT FADGNGTTAE VTKANDGSIT VKYNVKVADG LKLDGDKIVA Hia ATVASGTNVT FASGNGTTAT VTNGTDG.IT VKYDAKVGDG LKLDGDKLAA Hianm ETVTSGTNVT FASGKGTTAT VSKDDQGNIT VMYDVNVGDA LNVNQ..... 1750 1701 Hsf DTTVLTVAD. .....GKV TAPNNGDGKK FVDASGLADA LNKLSWTATA Hia DTTALTVNDG KNANNPKGKV ADVASTDEKK LVTAKGLVTA LNSLSWTTTA Hsf GKEGTGEVDP ANSAGQEVKA GDKVTFKAGD NLKIKQSGKD FTYSLKKELK Hia AEADGGTLD. GNASEQEVKA GDKVTFKAGK NLKVKQEGAN FTYSLQDALT Hianm G..SSGKVIS GNVSPSKGKM DETVNINAGN NIEITRNGKN I..DIATSMT .DLTSVEFKD ANGGTGSEST KITKDGLTIT PANGAGAAGA NTANTISVTK Hsf .GLTSITLGT GNNGA...KT EINKDGLTIT PANG...AGA NNANTISVTK Hia PQFSSVSLG. .....AGA D.APTLSV... HiaNm 1851 Hsf DGISAGNKAV TNVVSGLKKF GDGHTLANGT VAD.FEKHYD NAYKDLTNLD DGISAGGQSV KNVVSGLKKF GDANFDPLTS SADNLTKQND DAYKGLTNLD Hia HiaNm EKGADNN.PT VADNTAATVG DLRGLGWVIS ADKTTGEPNQ EYNAQVRNAN EKGTDKQTPV VADNTAATVG DLRGLGWVIS ADKTTGGST. EYHDQVRNAN \*\*\*\*\*\*\*\*\* \*\*\*\*\*\*\* \*\*\*\*\*\*\* \*\*\*\*\*\*\*\* 1951 EVKFKSGNGI NVSGKTLNGT RVITFELAKG EVVKSNEFTV KNADGSETNL EVKFKSGNGI NVSGKTVNGR REITFELAKG EVVKSNEFTV KETNGKETSL ....DGDAL NVGSK..... HiaNm 2050 VKVGDMYYSK EDIDPATSKP ...MTGKT..E KYKVENGKVV SANGSKTEVT Hsf VKVGDKYYSK EDIDLTTGQP KLKDGNTVAA KYQDKGGKVV SVTD.NTEAT Hia KDNKPV R.... HiaNm Hsf LTNKGSGYVT GNQVADAIAK SGFELGLADA AEAEKAFAES AKDKQLSKDK Hia ITNKGSGYVT GNQVADAIAK SGFELGLADE ADAKRAFDD. .KTKALSAGT HiaNm ITNVAPG....... Hsf AETVNAHDKV RFANGLNTKV SAATVESTDA NGDKVTTTFV KTDVELPLTQ TEIVNAHDKV RFANGLNTKV SAATVESTDA NGDKVTTTFV KTDVELPLTQ ...... HiaNm Hsf IYNTDANGNK I...VKKADG KWYELNADGT AS.NKEVTLG NVDANGKKVV IYNTDANGKK ITKVVKDGQT KWYELNADGT ADMTKEVTLG NVDSDGKKVV Hia .....VKEGD. ..

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## FIG. 6 cont'd

Hsf Hia HiaNm	2201 KVTENGADKW KDNDGKW	YYTNADGAAD YHAKADGTAD	KTKGEVSNDK KTKGEVSNDK	VSTDEKHVVR VSTDEKHVVS	2250 LDPNNQSNGK LDPNDQSKGK
	2251	1 12			2300
Hsf	GVVIDNVANG	FISATSTDAT	NGSOLYAVAK	GVTNLAGOVN	NLEGKVNKVG
Hia	CVVTDNVANG	DISATSTDAI	NGSOLYAVAK	GVTNLAGQVN	NLEGKVNKVG
HiaNm	. VTNVA		OLKGVA.	Q	NLNNRIDNVD
11 T CIVILI		••••			
•	2301	• 1			2350
Hsf	KRADAGTASA	LAASOLPOAT	MPGKSMVAIA	GSSYQGQNGL	AIGVSRISDN
Hia	KRADAGTASA	LAASOLPOAT	MPGKSMVAIA	GSSYQGQNGL	AIGVSRISDN
HiaNm	GNARAGIAOA	IATAGLVQAY	LPGKSMMAIG	GGTYRGEAGY	AIGYSSISDG
	2351		2378		
Hsf	GKVIIRLSGT	<b>TNSQGKTGVA</b>	AGVGYQW*		
Hia	GKVIIRLSGT	TNSQGKTGVA	AGVGYQW*		
HiaNm	GNWIIKGTAS	GNSRGHFGAS	ASVGYQW*		
HiaNm	GNWIIKGTAS	GNSRGHFGAS	ASVGYQW*		

h41

p20

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			12/13	•	••	•
FIG.	7					
• .	1				50	
220	MNEILRIIWN	CATMAGAAAA	ET TONUTED A	מ.דעמידאעייימס		
eg329	MNKIYRIIWN	CALMAMANA	ETIMUMADY FTIMUTUAL	CVM/MANUTY (		
pmc21	MNKIYRIIWN	SALNAWVVVS	ETIKMUIKKA	SWINDS AND A STANDARY	TITEVI AGUD	
HiaNm	MNKIYRIIWN	SALNAWVVVS	ELTRNHTKRA	SATVKTAVLA	TTTLWINGVY	
h15	MNKIYRIIWN	SALNAWVVVS	ELTRNHTKRA	SATVATAVLA	LTITENT AND A	
BZ10	MNKISRIIWN	SALNAWVVVS	ELTRNHTKRA	SATVATAVLA	TPPENIA OVA	•
bz198	MNKIYRIIWN	SALNAWVVVS	ELTRNHTKRA	SATVATAVLA	TUTEALAOWA	
eg327	MNKIYRIIWN	SALNAWVAVS	ELTRNHTKRA	SATVATAVLA	TPPENTANAV	
h38	MNKIYRIIWN	SALNAWVAVS	ELTRNHTKRA	SATVKTAVLA	TPPEALAGM	
h41	MNKIYRIIWN	SALNAWVAVS	ELTRNHTKRA	SATVKTAVLA	TLLFATVQAN	
p20	MNKIYRIIWN	SALNAWVVVS	ELTRNHTKRA	SATVATAVLA	TLLSATVQAN	
	51			•	100	
eq329	ANNE FORED	I.YI.DPVLRTV	AVLIVNSDKE	GTGEKEKVEE	NSDWAVYFNE	
pmc21	ANNE ECEED	I.YI.DPVORTV	AVLIVNSDKE	GTGEKEKVEE	NSDWAVYFNE	
HiaNm	PMMEDDBKKD	T.YT.DPVORTV	AVLIVNSDKE	GTGEKEKVEE	NSDWAVYFNE	
h15	מחת חיים	LYLEPVORTA	VVLSFRSDKE	GTGEKE.GTE	DSNWAVYFDE	
BZ10	מתח חדה	I.VI.EPVORTA	VVLSFRSDKE	GTGEKE.GTE	DSNWAVYFDE	. :
bz198	ממת חשת	T.VT.EPVORTA	VVLSFRSDFE	GTGEKE.GTE	DSNWAVYFDE	
eg327	ממטייים מישה	T.VT.FDV/\PTA	VVLSFRSDKE	GTGEKE.VTE	DSNWGVYFDK	
eg327 h38	TIDDDD	ERITED/VINCE	LVLQFMIDKE	GNGENE.STG	NIGWSIYYDN	•
h41	WIDEDEE	EETEL ALUDA	VVGSIQASME	GSVELETI	SLSMTNDS	
p20	WIDEDEE	EEDESVARSA	LVLQFMIDKE	GNGEIE.STG	DIGWSIYYDD	
pzu	AIDIDED	EEHED VALOR	B4222			٠.
	101		•		150	
eg329	KGVLTA.REI	TLKAGDNLKI	ко	NGTNETYS	TVVDTIDITS	
pmc21	KGVLTA.REI	TLKAGDNLKI	KQ	NGTNFTYS	TKKDFIDFIS	
HiaNm	KGVLTA.REI	TLKAGDNLKI	KQ	NGTNFTYS	PKKDPIDPIS	
h15	KRVLKA GAI	TLKAGDNLKI	KONTNENTNE	NTNDSSETYS	PKKDFLDFLS	,
BZ10	KRVLKA.GAI	TLKAGDNLKI	KQNTNENTNE	NTNDSSETYS	PKKDPIDIIS	
bz198	KRVLKA.GAI	TLKAGDNLKI	KQNINE	NTNDSSETYS	PKKDPLDPIS	
eg327	KGVLTA.GTI	TLKAGDNLKI	KQNTNE	NTNASSETYS	FKKDFLDFL2	,
h38	HNTLHG. ATV	TLKAGDNLKI	KONTNKNTNE	NTNDSSETYS	TKKDFIDELS	,
h41	KEFVDPYIVV	TLKAGDNLKI	KQNTNE	NTNASSETYS	PKKDLIGHTN	,
p20	HNTLHG.ATV	TLKAGDNLKI	KQ	SGKDFTYS	LKKELKDLTS	,
	151	·		•	200	
~~330	WOMENT CECT	NCNKUNTTSI	TKGLNFAKET	AGTNGDTTVH	LNGIGSTLTI	)
eg329 pmc21	TOWERT CEC!	NCNKVNTTSI	) TKGINFAKET	' AGTNGDTTVH	TWETCOIPTI	•
PMC21 HiaNm	MORPHY CECT	MCNKVNTTSI	) TKGINFAKET	' AGTNGDTTVH	TWGTG21T1	J
nianm h15	TIRMENT CECT	MCMKUMTTSI	TYCLNFAKET	r AGTNGDPTVE	[ PMCTC21TI	ע
BZ10	ייים דער פער	NCNKVNTTSI	TKGINFAKET	' AGTNGDPTVE	TWGTG21711	U
bz198	TRANSPET CEC	N NICHTENNITTEI	n TKGINFAKET	r AGTNGDPTVI	TMGTGSTTT	υ
eg327	TEMPET CEC	NGMWWITTS1	D TKGINFAKK	r AETNGDTTV:	TMCTC2171	υ
eg327 h38	TEMPUT CEC	N NCNEVNTTS	D TKCINFAKE	r agtngdtivi	TWGTGSIDI	υ
N30	A ET EVTISE (2)	NCKKVNITS!	D TKGLNFAKE	r agtngdttvi	I LNGIGSTLT	D

VETEKLSFGA NGKKVNIISD TKGLNFAKET AGTNGDTTVH LNGIGSTLTD

VETEKLSFGA NGNKVNITSD TKGLNFAKET AGTNGDPTVH LNGIGSTLTD

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# FIG. 7 cont'd

				•		• •
		201		•		250
			VTNDNVTDDE	KKRAASVKDV	LNAGWNIKGV	KPGTTASD
	pmc21	TLLNTGATTN	VTNDNVTDDE	KKRAASVKDV	LNAGWNIKGV	KPGTTASD
	HiaNm	TLLNTGATTN	VTNDNVTDDE	KKRAASVKDV	LNAGWNIKGV	KPGTTASD
	h15	TI.I.NTGATTN	VTNDNVTDDE	KKRAASVKDV	LNAGWNIKGV	KPGTTASD
	BZ10	TLLNTGATTN	VTNDNVTDDE	KKRAASVKDV	LNAGWNIKGV	KPGTTASD
	bz198	TT.T.NTGATTN	VTNDNVTDDE	KKRAASVKDV	LNAGWNIKGV	KPGTTASD
	eg327	TILINTGATTN	VTNDNVTDDE	KKRAASVKDV	LNAGWNIKGV	KPGTTASD
	h38	TT.T.NTGATTN	VTNDNVTDDK	KKRAASVKDV	LNAGWNIKGV	KPGTTASD
	h41	MT.T.NTCATTN	VINDNVTDDE	KKRAASVKDV	LNAGWNIKGV	KPGTTASD
	p20	TT.AGSSASHV	DAGNOSTHY.	.TRAASIKDV	LNAGWNIKGV	KTGSTTGQSE
	p20	1111000110111				
		251				300
	eq329	MAN EADEADA	VEFT.SAUTKT	TTVNVESKDN	GKKTEVKIGA	KTSVIKEKDG
	pmc21		VEFLSADTKT		GKKTEVKIGA	KTSVIKEKDG
	HiaNm	MADEARTADA	VEFLSADTKT			KTSVIKEKDG
	h15	MADIAKIDAADA	VEFLSADTKT	TTVNVESKDN		KTSVIKEKDG
	BZ10	MADIANIE	VEFT.SADTKT	TTVNVESKDN	GKRTEVKIGA	KTSVIKEKDG
	bz198	MADEAUTIDE	VEFT.SADTKT	TTVNVESKDN	GKKTEVKIGA	KTSVIKEKDG
•	eg327	MADEABAAAA	VEFT.SADTKT	TTVNVESKDN	GKRTEVKIGA	KTSVIKEKDG :
	eg327 h38	יועראייואטיבערעטיי	VEFT.SADTKT	TTVNVESKDN	GKRTEVKIGA	KTSVIKEKDG
	h41	MADEAUGAAAA	VEFLSADTKT	TTVNVESKDN	GKKTEVKIGA	KTSVIKEKDG
	p20	MADEARTADE	VEFLSADTKT	TTVNVESKDN	GKRTEVKIGA	KTSVIKEKDG
	. p20	. MADT AWITET	<b>V</b>			
		301				350
	eq329	KINTCKDKGF	NGSSTDEGE	LVTAKEVIDA	VNKAGWRMKI	TTANGQTGQA
	pmc21	KTAVTGKDKGF	NGSSTDEGEG	LVTAKEVIDA	vnkagwrmki	TTANGQTGQA
	HiaNm	KINTCKDKCE	NGSSTDEGE	LVTAKEVIDA	. VNKAGWRMK'I	TTANGQTGQA
	h15	KIATGKGKDE	NGSSTDEGE	LVTAKEVIDA	\ VNKAGWRMKI	TTANGQTGQA
	BZ10	KTAMCKCKCK	NGSSTDEGE	LVTAKEVIDA	<b>\ VNKAGWRMK</b> !	TTANGQTGQA
	bz198	KINTCKCKDE	NGSSTDEGE	LVTAKEVIDA	<b>VNKAGWRMK</b>	TTANGQTGQA
	eg327	KTAPICKDKCE	NDSSTDKGE	<b>LVTAKEVID</b>	A VNKAGWRMK	TTANGQTGQA
	h38	KT.VTGKGKGI	NGSSTDEGE	3 LVTAKEVIDA	a vnkagwrmk:	r TTANGQTGQA
	h41	KINTCKCKCI	R NGSSTDEGE	3 LVTAKEVIDA	a vnkagwrmk:	r Tranguigua
	p20	KLVTGKGKG	NGSSTDEGE	G LVTAKEVID	a vnkagwrmk	r ttangqtgqa
	Pav			ta de la seguir	A character of the	· · · · · · · · · · · · · · · · · · ·
		351				400
	eg329	DKFETVTSG	T NVTFASGKG	T TATVSKDDQ	G NITVMYDVN	V GDALNVNQLQ
	pmc21	DKFETVTSG		TATVSKDDO	G NITVMYDVN	V GDALNVNQLQ
	HiaNm	DKFETVTSG		T TATVSKDDQ	G NITVMYDVN	V GDALNVNQLQ
	h15	DKFETVTSG		ጥ ጥልጥህናዘገነበር	G NITVKYDVN	V GDALNVNQLQ
	BZ10	DKFETVTSG		ጥ ጥልጥህናለከክር	G NITVKYDVN	V GDALNVNQLQ
	bz198	DKFETVTSG		T TATVSKIDO	G NITVKYDVN	V GDALNVNQLQ
	eg327	DKFETVTSG		T TATVSKIDO	G NITVMYDVN	V GDALNVNQLQ
	h38	DKFETVTSG		TATVSKDDO	G NITVKYDVN	ΓV GDALNVNQLQ
	h41	DKFETVTSG	m vameraschic	TATVSKDDO	G NITVKYDVN	A CDYTNANOTO
	p20	DKFETVTSG	T KVTFASGNO	TATVSKDDQ	OG NITVKYDVN	N CDATNANOTO
	P20					-

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#### FIG. 7 cont'd

		401				450
•	eg329	NSGWNLDSKA	VAGSSGKVIS	GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
•	pmc21	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
	HiaNm	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
	h15	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
٠,	B210	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
	bz198	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
	eq327	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
	h38	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
	h41	NSGWNLDSKA		<b>GNVSPSKGKM</b>	DETVNINAGN	NIEITRNGKN
	p20	NSGWNLDSKA		GNVSPSKGKM	DETVNINAGN	NIEITRNGKN
	pro					
		451				500
٠	eg329	IDIATSMTPO	FSSVSLGAGA	DAPTLSVDGD	.ALNVGSKKD	NKPVRITNVA
	pmc21	IDIATSMTPO	<b>FSSVSLGAGA</b>	DAPTLSVDGD		NKPVRITNVA
	HiaNm	IDIATSMTPO	FSSVSLGAGA	DAPTLSVDGD		NKPVRITNVA
	h15	IDIATSMTPO	FSSVSLGAGA	DAPTLSVDDE		NKPVRITNVA
	BZ10	TDIATSMTPO	FSSVSLGAGA	DAPTLSVDDE		nkpvritnva
•	bz198	IDIATSMAPO	FSSVSLGAGA	DAPTLSVDDE		nkpvritnva
٠,	eg327	IDIATSMTPO	FSSVSLGAGA	DAPTLSVDDE		nkpvritnva
	h38	IDIATSMTPO	FSSVSLGAGA	DAPTLSVDDK	GALNVGSKDA	NKPVRITNVA
	h41	TDTATSMTPO	FSSVSLGAGA	DAPTLSVDDE	GALNVGSKDA	NKPVRITNVA
	p20	IDIATSMTPO	FSSVSLGAGA	DAPTLSVDDE	GALNVGSKDA	NKPVRITNVA
	PDU		, –			
		501		:		550
	eg329	PGVKEGDVTN	VAQLKGVAQN	LNNRI DNVDG	NARAGIAQAI	ATAGLVQAYL
	pmc21	PGVKEGDVTN	VAQLKGVAQN	INNRIDAVDG	: NARAGIAQAI	ATAGLVQAYL
	HiaNm	PGVKEGDVTN	VAQLKGVAQN	LNNRIDNVD	NARAGIAQAI	ATAGLVQAYL
	h15	PGVKEGDVTN	VAQLKGVAQN	LNNRIDNVD	, naragiaqai	ATAGLAQAYL
	BZ10	PGVKEGDVTN	VAQLKGVAQN	I INNRTDNVD	: NARAGIAQAI	ATAGLAQAYL
	bz198	PGVKEGDVTN	VAQLKGVAQN	I INNRIDNVDO	S NARAGIAQAI	ATAGLVQAYL
	eg327	PGVKEGDVT	VAQLKGVAQN	TANNIT DNVD	S NARAGIAQAI	ATAGLVQAYL
	h38	PGVKEGDVTI	VAQLKGVAQN	I LNNRIDNVDO	s naragiaqaj	ATAGLVQAYL
	h41	PGVKEGDVTI	N VAQLKGVAQI	I LNNRIDNVN	s naragiaqai	ATAGLVQAYL
	p20	PGVKEGDVTI	N VAQLKGVAQI	I LNNRIDNVN	s naragiaqai	ATAGLAQAYL
						600
•		551				• • •
	eg329	PGKSMMAIG	G GTYRGEAGY	A IGYSSISDG	G NWIIKGTAS	NSRGHFGASA
	pmc21	DOVEMBATO	C COVDERAGY	A TGYSSISDG	G NWIIKGTAS	2 NOKGUEGUDU
	HiaNm	- DOWNOOTO	へ へがくりになるにく	N TCVSSTSDG	G NWIIKGTAS	6 NOKONEGROA
١	h15	DOVENOM TO	C COVDERNEY	A TGYSSTSDT	G NWV1KGTAS	G NOKODEGNON
	BZ10	DOVEMBATO	C CTVDCRACY	A TGYSSISDT	G NWVIKGIAS	6 Novementary
	bz198	nevenna Te	C DEVDCEAGY	A TGYSSISDG	G NWIIKGIAS	G NOKGHE GROW
	eg327	PGKSMMAIG	C CTVDCFACY	A TGYSSTSDG	G NWIIKGIAS	G Makaurawa
	h38	PGKSMMAIG	~ ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	A TCVSSTSDG	G NWIIKGIAS	G Nokentaron
	h41	DOVEMAN TO	C COVICEACY	A TGYSSISAG	G NWIIKGTAS	G NSRGHEGASA
	p20		G GTYLGEAGY	A IGYSSISDT	G NWVIKGTAS	G NSRGHFGTSA
	F0					

WO 99/31132 PCT/AU98/01031

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#### FIG. 7 cont'd

601 eg329 pmc21 HiaNm SVGYQW\* SVGYQW\* SVGYQW\* h15 BZ10 bz198 SVGYQW\* SVGYQW\* eg327 h38 SVGYQW\* h41 p20 SVGYQW\*

#### SEQUENCE LISTING

<110> Peak, Ian R. (U.S. only) Jennings, Michael P. (U.S. only) Moxom, Edward R. (U.S. only) University of Queensland (except U.S.) Isis Innovations Limited (except U.S.) <120> Novel surface antigen <130> Neisseria meningitidis HiaNm antigen <140> PCT/AU98/01031 <141> 1998-12-14 <150> GB 9726398.2 <151> 1997-12-12 <160> 31 <170> PatentIn Ver. 2.0 <210> 1 <211> 2308 <212> DNA <213> Neisseria meningitidis <220> <221> CDS <222> (276)..(2054) <400> 1 gaaaaaccac aggaatttat cagcaaaaac agaaacccca ccgccgtcat tcccgcaaaa 60 gcgggaatcc agacccgtcg gcacggaaaa cttaccgaat aaaacagttt ccttagattc 120 cacgtcccag attcccgcct tcgcggggaa tgacgagatt ttaagttggg ggaatttatc 180 agaaaacccc caacccccaa aaaccgggcg gatgccgcac catccgcccc caaaccccga 240 tttaaccatt caaacaaacc aaaagaaaaa acaaa atg aac aaa ata tac cgc Met Asn Lys Ile Tyr Arg 341 atc att tgg aat agt gcc ctc aat gcc tgg gtc gtc gta tcc gag ctc Ile Ile Trp Asn Ser Ala Leu Asn Ala Trp Val Val Ser Glu Leu 15 10 aca cgc aac cac acc aaa cgc gcc tcc gca acc gtg aag acc gcc gta 389 Thr Arg Asn His Thr Lys Arg Ala Ser Ala Thr Val Lys Thr Ala Val 25 . ttg gcg aca ctg ttg ttt gca acg gtt cag gca agt gct aac aat gaa 437 Leu Ala Thr Leu Leu Phe Ala Thr Val Gln Ala Ser Ala Asn Asn Glu 40 45 aga cca aga aag aaa gat tta tat tta gac ccc gta caa cgc act gtt Arg Pro Arg Lys Lys Asp Leu Tyr Leu Asp Pro Val Gln Arg Thr Val 55 60 533 gcc gtg ttg ata gtc aat tcc gat aaa gaa ggc acg gga gaa aaa gaa Ala Val Leu Ile Val Asn Ser Asp Lys Glu Gly Thr Gly Glu Lys Glu 75

														3,		
aaa Lys	gta Val	gaa Glu	gaa Glu 90	aat Asn	tca Ser	gat Asp	tgg Trp	gca Ala 95	gta Val	tat Tyr	ttc Phe	aac Asn	gag Glu 100	aaa Lys	gga Gly	581
gta Val	cta Leu	aca Thr 105	gcc Ala	aga Arg	gaa Glu	atc Ile	acc Thr 110	ctc Leu	aaa Lys	gcc Ala	ggc Gly	qeA	aac Asn	ctg Leu	aaa Lys	629
atc Ile	aaa Lys 120	caa Gln	aac Asn	ggc Gly	aca Thr	aac Asn 125	ttc Phe	acc Thr	tac Tyr	tcg Ser	ctg Leu 130	aaa Lys	aaa Lys	gac Asp	ctc Leu	677
aca Thr 135	gat Asp	ctg Leu	acc Thr	agt Ser	gtt Val 140	gga Gly	act Thr	gaa Glu	aaa Lys	tta Leu 145	tcg Ser	ttt Phe	agc Ser	gca Ala	aac Asn 150	725 <sub></sub>
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aac Asn	gta Val 200	acc Thr	aac Asn	gac Asp	aac Asn	gtt Val 205	acc Thr	gat Asp	gac Asp	gag Glu	aaa Lys 210	aaa Lys	cgt Arg	gcg Ala	gca Ala	917
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gca Ala	aaa Lys	gaa Glu	gtg Val	att Ile 315	Asp	gca Ala	gta Val	aac Asn	aag Lys 320	Ala	ggt Gly	tgg Trp	aga Arg	atg Met 325	Lys	1253
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Val	Thr	Ser	Gly	Thr	Asn	Val	Thr	Phe	Ala	Ser	Gly	Lys	Gly	Thr	Thr	
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Ala	Thr	Val	Ser	Lys	Asp		G1n	Gly	Asn	Ile <sub>.</sub>		Val	Met	Tyr	Asp	
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	Asn	Val	Gly	Asp		Leu	Asn	Val					Asn	Ser		
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agc	ggc	aat	gtt	tcg	ccg	agc	aag	gga	aag	atg	gat	gaa	acc	gtc	aac	1541
ser	Gly	Asn		ser	PIO	ser	гÀа		гая	met	ASP	GIU		AGI	ASII	
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	atc Ile															11377
Asp	440	HIG	TIIL	Ser	Het	445	FIU	GIII	FILE	Der	450	Val	261	Deu	329	
	440		2			443				1.0	-130	(a)	1.75	Ċ.	1947	<i>્રમાં</i> (તે ફિલ્
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ycy N1a	Gly	ycy N1a	) den	Als	Pro	Thr	LLU	Car	Val	Den	'61 v	Zen	'Ala	T.em	Asn	
455	СТУ	WIG	nap	<b>u</b> ra	460	1111	Lea		v.a.i	465	GLY	nsp	nia	DCu	470	
433					400		-			403						
atc	ggc	anc	220	aan	nac	aac	222	CCC	atc	CUC	att	acc	aat	atc	acc	1733
Val	Gly	Sor	Tue	T.ve	Agn	Asn	Lvg	Pro	Val	Ara	Tle	Thr	Asn	Val	Ala	
AGT	GLY	Jer	БуЗ	475	лор	non,	בעם	110	480	1119	110	****		485		
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cca	aac	att	aaa	gag	aaa	gat	att	aca	aac	atc	σса	caa	ctt	aaa	ggc	1781
	Gly															2.1.1
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ata	gcg	caa	aac	tta	aac	aac	cqc	atc	qac	aat	ata	qac	qqc	aac	gcg	1829
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		505					510		•	, .		515	:			31
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cgt	gcg	ggc	atc	gcc	caa	gcg	att	gca	acc	gca	ggt	ctg	gtt	cag	gcg	1877
Arg	Ala	Gly	Ile	Ala	Gln	Ala	Ile	Ala	Thr	Ala	Gly	Leu	Val	Gln	Ala	
•	520	-	:			525	٠.		,	٠.	530		* ;	: .	33.4	
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aat	tgg	att	atc	aaa	ggc	acg	gct	tcc	ggc	aat	tcg	cgc	ggc	cat	ttc	2021
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Ala Ser Ala Asn Asn Glu Arg Pro Arg Lys Lys Asp Leu Tyr Leu Asp 50 55 60

Pro Val Gln Arg Thr Val Ala Val Leu Ile Val Asn Ser Asp Lys Glu 65 70 75 80

Gly Thr Gly Glu Lys Glu Lys Val Glu Glu Asn Ser Asp Trp Ala Val 85 90

Tyr Phe Asn Glu Lys Gly Val Leu Thr Ala Arg Glu Ile Thr Leu Lys 100 105 110

Ala Gly Asp Asn Leu Lys Ile Lys Gln Asn Gly Thr Asn Phe Thr Tyr 115 120 125

Ser Leu Lys Lys Asp Leu Thr Asp Leu Thr Ser Val Gly Thr Glu Lys 130 135 140

Leu Ser Phe Ser Ala Asn Gly Asn Lys Val Asn Ile Thr Ser Asp Thr 145 150 155 160

Lys Gly Leu Asn Phe Ala Lys Glu Thr Ala Gly Thr Asn Gly Asp Thr 165 170 175

Thr Val His Leu Asn Gly Ile Gly Ser Thr Leu Thr Asp Thr Leu Leu 180 185 190

Asn Thr Gly Ala Thr Thr Asn Val Thr Asn Asp Asn Val Thr Asp Asp 195 200 205

Glu Lys Lys Arg Ala Ala Ser Val Lys Asp Val Leu Asn Ala Gly Trp 210 215 220

Asn Ile Lys Gly Val Lys Pro Gly Thr Thr Ala Ser Asp Asn Val Asp 225 230 235 240

Phe Val Arg Thr Tyr Asp Thr Val Glu Phe Leu Ser Ala Asp Thr Lys 245 250 255

Thr Thr Thr Val Asn Val Glu Ser Lys Asp Asn Gly Lys Lys Thr Glu 260 265 270

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Leu	Val 290	Thr	Gly	Lys	Asp	Lys 295	Gly	Glu	Asn	Gly	Ser 300	Ser	Thr	Asp	Glu
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Ala	Gly	Trp	Arg	Met 325	Lys	Thr	Thr	Thr	Ala 330	Asn	Gly	Gln	Thr	Gly 335	Gln
Ala	Asp	Lys	Phe 340	Glu	Thr	Val	Thr	Ser 345	Gly	Thr	Asn	Val	Thr 350	Phe	Ala
Ser	Gly	Lys 355	Gly	Thr	Thr	Ala	Thr 360	Val	Ser	Гуз	Asp	Asp 365	Gln	Gly	Asn
Ile	Thr 370	Val	Met	Tyr	Asp	Val 375	Asn	Val	Gly	Asp	<b>Ala</b> 380	Leu	Asn	Val	Asn
Gln 385	Leu	Gln	Asn	Ser	Gly 390	Trp	Asn	Leu	Asp	Ser 395	Lys	Ala	Val	Ala	Gly 400
Ser	Ser	Gly	Lys	Val 405	Ile	Ser	Gly	Asn	Val 410	Ser	Pro	Ser	Lys	Gly 415	Lys
Met	Asp	Glu	Thr 420	Val	Asn	Ile	Asn	Ala 425	Gly	Asn	neA	Ile	Glu 430	Ile	Thr
Arg	Asn	Gly 435	Lys	Asn	Ile	Asp	Ile 440	Ala	Thr	Ser	Met	Thr 445	Pro	Gln	Phe
Ser	Ser 450	Val	Ser	Leu	Gly	Ala 455	Gly	Ala	Asp	Ala	Pro 460	Thr	Leu	Ser	Val
Asp 465	Gly	Asp	Ala	Leu	Asn 470	Val	Gly	Ser	Lys	Lys 475	Asp	Asn	Lys	Pro	Val 480
Arg	Ile	Thr	Asn	Val 485	Ala	Pro	Gly	Val	Lys 490		Gly	Asp	Val	Thr 495	Asn
Val	Ala	Gln	Leu 500	Lys	Gly	Val	Ala	Gln 505	Asn	Leu	Asn	Asn	Arg 510	Ile	Asp
Asn	Val	Asp 515	Gly	Asn	Ala	Arg	Ala 520	Gly	Ile	Ala	Gln	Ala 525	Ile	Ala	Thr
Ala	Gly 530	Leu	Val	Gln	Ala	Tyr 535		Pro	Gly	Lys	Ser 540	Met	Met	Ala	Ile
Gly 545	Gly	Gly	Thr	Tyr	Arg 550	Gly	Glu	Ala	Gly	Tyr 555		Ile	Gly	Tyr	Ser 560
Ser	Ile	Ser	Asp	Gly 565		Asn	Trp	Ile	Ile 570		Gly	Thr	Ala	Ser 575	Gly
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<sup>&</sup>lt;213> Neisseria meningitidis

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ggcacgggag	aaaaagaaaa	agtagaagaa	aattcagatt	gggcagtata	tttcaacgag	300
aaaggagtac	taacagccag	agaaatcacc	ctcaaagccg	gcgacaacct	gaaaatcaaa	360
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catttcggtg	cttccgcatc	tgtcggttat	: cagtggtaa			1779

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gtc Val	-	-					_			acc Thr					_	96
										ttg Leu						144
							Asp			tta Leu	Glu	Pro	Val		Arg	192
	-	-	-	-	•		_		-	aaa Lys 75						240
aaa Lys	gaa Glu	ggt Gly	aca Thr	gaa Glu 85	gat Asp	tca Ser	aat Asn	tgg Trp	gca Ala 90	gta Val	tat Tyr	ttc Phe	gac Asp	gag Glu 95	aaa Lys	288
agaʻ Arg	gta Val	cta Leu	aaa Lys 100	gcc Ala	gga Gly	gca Ala	atc Ile	acc Thr 105	ctc Leu	aaa Lys	gcc Ala	ggc Gly	gac Asp 110	aac Asn	ctg Leu	336
							_			aat Asn	-					384
agt Ser	agc Ser 130	ttc Phe	acc Thr	tac Tyr	tcc Ser	ctg Leu 135	aaa Lys	aaa Lys	gac Asp	ctc Leu	aca Thr 140	gat Asp	ctg Leu	acc Thr	agt Ser	432
										aac Asn 155						480
					Lys					gcg Ala						528
acg Thr	aac Asn	ggc Gly	gac Asp 180	Pro	acg Thr	gtt Val	cat His	ctg Leu 185	aac Asn	ggt Gly	atc Ile	ggt Gly	tcg Ser 190	Thr	ttg Leu	576
	-	_	Leu	_				Ala		aca Thr		_	Thr		gac Asp	624
		Thr					Lys			gca Ala		Val				672
tta	aac	gca	ggc	tgg	aac	att	aaa	ggc	gţt	aaa	ccc	ggt	aca	aca	gct	720

							,	·		•• •••	M.	3 · · · ;	33 (AV)			
Leu 225	Asn	Ala	Gly	Trp	Asn 230	Ile	Lys	Gly	Val	Lys 235	Pro	Gly	Thr	Thr	Ala 240	
				gat												768
Ser	Asp	Asn	Val	Asp	Phe		Arg			Asp	Thr	Val	Glu		Leu	
				245	1.			. ,	250	,	• ;	•	9 H	255	$A^{\prime}$ , $A^{\prime}$ ,	
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													Lys		Asn	
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Gly	Lys	Arg	Thr	Glu	Val	Lys	Ile	Ğĺy	Ala	Lys	Thr	Ser	Val	Ile	Lys	
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asa	222	gac	aat	ааσ	ttα	att	act	aat:	aaa	ggc.	aaa	aac	σaσ	aat	ggt	912
Glu	Lys	Asp	Gly	Lys	Leu	Val	Thr	Gly	Lys	Ğly	Lys	Gly	Ğlu	Asn	Gly	-
	290	_	_			295		•			300					
+a+	+	262	~~~	~~~	aac'	~	aac	++=	ata	act	oca.	222	gaa	ata	att	960
Ser	Ser	Thr	Asp	Glu	Glv	Glu	Glv	Leu	Val	Thr	Ala	Lys	Glu	Val	Ile	
305		1.6	•		310		(1)		, <i>(ii</i> );	315	4.71		13.7		320	
				: ::							$H_{ijk}$	1000	<b>7.74</b> 27	() ()	ker Cit	1008
gat	gca	gta Val	aac Asn	aag Lys	Ala	Glv	Tro	Ara	Met	Lvs	Thr	Thr	Thr	Ala	Asn	1000
шр	· .			325		,	ŢĒ.	3	330				jak,	335	1,11	
	· ·						-ij			111					1	1056
ggt	Caa	aca	ggt	caa Gln	gct	gac	aag Lvs	Phe	Glu	Thr	gtt Val	Thr	Ser	ggc Glv	Thr	1056
GIY.	GIII	1111	340	GIII	ATG.	,rup		345	<b>0.1</b> 0		***		350	02,		
						•	ž		:					:	٠.	1104
aaa	gta	acc	ttt	gct. Ala	agt	ggt	aat	ggt	aca	act	gcg	act	gta	agt	aaa Lvs	1104
гàз	VAI	355	File	AIG	261	GLY	360	GLY	****		71_0	365			2,0	•:
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gat	gat	caa	ggc	aac Asn	atc	act	gtt	aag	tat	gat	gta	aat	gtc	ggc	gat	1152
Asp	370	GIII	GIY	ASII	116	375	AGT	цуз	TYL	nap	380	NO!!	VIII	Q_y	p	
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				aat Asn											tcc	1200 y
385	Leu	ASII	. vai	ASII	390		9111		Ser		ILD	ASII	Deu	nsp	400	
	٠	•				<b>'</b> .		'. :	1.17		: '					
aaa	gcg	gtt	gca	ggt	tct	tcg	ggc	aaa	gtc	atc	agc	ggc	aat	gtt	tcg	1248
ràs	AIa	vaı	AIa	405	Ser	Ser	СТА	гÃ2	410	116	Ser	GLY	ASII	415	Ser	
									• .•		٠.					1
ccg	agc	aag	gga	aag	atg	gat	gaa	acc	gtc	aac	att	aat	gcc	ggc	aac	1296
Pro	ser	гÀЗ	420		Met	Asp	GIU	425		ASII	116	ASII	430		Asn	
	:. ·	:			1.5							: : :	:	. • . • .		
aac	atc	gag	att	acc	cgc	aac	ggc	aaa	aat	atc	gac	atc	gcc	act	tcg	1344
Asn	Ile	G1u 435		Thr	Arg	Asn	G1y 440		Asn		Asp	11e		Int	Ser	V 2.7 VV
		133							1 '1		 3					
atg	acc	ccg	caa	ttt	tcc	agc	gtt	tcg	cto	ggc	gcg	ggg	gcg	gat	gcg	1392
Met			Gln	Phe	Ser	Ser 455		Ser	Leu	Gly	Ala 460		/ Ala	Asp	Ala	
	450					400	, ; .		• • • •		300	•				
															aag	1440
		Leu	Ser	Val	_	_	Glu	Gly	Ala			Val	Gly	Ser	Lys	•
465					470	ı	; .		**··*	475	•				480	
gat	gcc	aac	aaa	ccc	gto	cgc	att	acc	aat	gto	gco	ccg	ggo	gtt	aaa	1488
Asp	Ala	Asn	Lys	Pro	Val	Arg	Ile	Thr	Asn	Val	Ala	Pro	G1,	/ Val	. Lys	1

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		• •		485				:	490			• • •	• ;	495		
		gat Asp														1536
		aac Asn 515														1584
		gcg Ala														1632
		atg Met														1680
tac Tyr	gcc Ala	atc Ile	ggc Gly	tac Tyr 565	tcg Ser	agc Ser	att Ile	tct Ser	gac Asp 570	Thr	ggg Gly	aat Asn	tgg Trp	gtt Val 575	atc Ile	1728
aag Lys	ggc Gly	acg Thr	gct Ala 580	tcc Ser	ggc Gly	aat Asn	tcg Ser	cgc Arg 585	ggt Gly	cat His	ttc Phe	ggt Gly	act Thr 590	tcc Ser	gca Ala	1776
		ggt Gly 595							<i>i</i>	·; .			•		÷	1797
<21	l> 5: 2> <b>P</b> 1		eria	men	ingit	tidi:	s.			,						
	)> 5 <b>As</b> n	Lys	Ile	Ser 5		Ile	Ile	Trp	Asn 10		Ala	Leu	Asn	Ala 15	Trp	
Val	Val	Val	Ser 20	Glu	Leu	Thr	Arg	Asn 25	His	Thr	Lys	Arg	Ala 30	Ser	Ala	•
Thr	٠.	Ala 35	Thr	Ala	Val	Leu	Ala 40	Thr	Leu	Leu	Phe	Ala 45		Val	Gln	-
Ala	Asn 50		Thr	Asp	Asp	Asp 55	_	Leu	Tyr	Leu	Glu 60		Val	Gln	Arg	
Thr 65	Ala	Val	Val	Leu	Ser 70	Phe	Arg	Ser		Lys 75		Gly	Thr	Gly	Glu 80	
Lys	Glu	Gly	Thr	G1u 85		Ser	Asn	Trp	Ala 90		Tyr	Phe	Asp	Glu 95		
Arg	Val	Leu	Lys 100		Gly	Ala	Ile	Thr 105		Lys	Ala	Gly	Asp 110		Leu	• •
Lys	Ile	Lys 115	Gln	Asn	Thr	neA	Glu 120		Thr	Asn	Glu	Asn 125		Asn	Asp	
Ser	Ser 130		Thr	Tyr	Ser	Leu 135		Lys	Asp	Leu	Thr 140		Leu	Thr	Ser	

Val Glu Thr Glu Lys Leu Ser Phe Gly Ala Asn Gly Asn Lys Val Asn

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	Asn	Val 210	Thr	Asp	Asp	Glu	Lys 215	Lys	Arg	Ala	Ala	Ser 220	Val	Lys	Asp	Val
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				Thr 260					265					270		
	_	_	275	Thr				280					285			
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	-			Gly 340					345					350		
	-		355	Phe				360					365			
	-	370		Gly			375					380				
	385			Val		390					395					400
	-				405					410					415	
				Gly 420					425					430		
			435					440					445			
		450		Gln			455					460				
	465			Ser		470					475					480
	Asp	Ala	Asn	Lys	Pro		Arg	Ile	Thr	Asn 490		Ala	Pro	Gly	Val	

					•			•					. *		•	
Glu	Gly	Asp	Val 500	Thr	Asn	Val	Ala	Gln 505	Leu	Lys	Gly	Val	Ala 510	Gln	Asn	
Leu	Asn	Asn 515	Arg	Ile	Asp	Asn	Val 520	Asp	Gly	Asn	Ala	Arg 525	Ala	Gly	Ile	• • •
Ala	Gln 530	Ala	Ile	Ala	Thr	Ala 535	Gly	Leu	Ala		Ala 540	Tyr	Leu	Pro	Gly	
Lys 545	Ser	Met	Met	Ala	Ile 550	Gly	Gly	Gly	Thr	Tyr 555	Arg	Gly	Glu	Ala	Gly 560	
Tyr	Ala	Ile	Gly	Tyr 565	Ser	Ser	Ile	Ser	Asp 570	Thr	Gly	Asn	Trp	Val 575	Ile	
Lys	Gly	Thr	Ala 580	Ser	Gly	Asn	Ser	Arg 585	Gly	His	Phe	Gly	Thr 590	Ser	Ala	
Ser	Val	Gly 595	Tyr	Gln	Trp		į		<b>'</b>	. :						
	0> 6 1> 1 <sup>-</sup>	785			:	ι <b>5</b> .			· .		. '	· 	<i>.</i>	•		. 47 A.
	2> DI 3> No		eria	men	ingi	tidi	<b>S</b> .		:							
	0> 1> .Cl 2> ()		(178	5)		٠.							•			
-401	0> 6								:	٠						
atq	aac	aaa Lys	ata Ile	tac Tyr 5	Arg	atc Ile	att Ile	tgg Trp	aat Asn 10	Ser	gcc Ala	ctc Leu	aat Asn	gcc Ala 15	tgg Trp	48
gtc Val	gtc Val	gta Val	tcc Ser 20	gag Glu	ctc Leu	aca Thr	cgc Arg	aac Asn 25	cac His	acc Thr	aaa Lys	cgc Arg	gcc Ala 30	tcc Ser	gca Ala	96
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gcg Ala	Asn	gct	acc	gat Asp	gac Asp	Asp	gat	tta Leu	tat Tyr	tta Leu	Glu	CCC	gta Val	caa Gln	cgc Arg	192
act	50 gct	gtc	gtg	ttg	agc	55 ttc	cgt Ara	tcc	gat	aaa Lvs	gaa Glu	ggc G1 v	acg Thr	gga Glv	gaa Glu	240
65		٠.			70		*.	•	• • • •	75					80	288
Lys	gaa Glu	Gly	Thr	Glu 85	Asp	Ser	Asn	Trp	Ala 90	Val	Tyr	Phe	Asp	Glu 95		
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tac	tcc	cta	aaa		gac	cto	aca	gat	cta	acc	aσt	att	σаа	act	gaa	432

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Tyr	Ser 130	Leu	Lys	Lys	Asp	Leu 135	Thr	Asp	Leu	Thr	Ser 140	Val	Glu	Thr	Glu	
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	acg Thr										Leu			Thr		576
-	aat Asn							-			_		_		_	624
	gag Glu 210									Asp						672
tgg Trp 225	aac Asn	att Ile	aaa Lys	ggc Gly	gtt Val 230	aaa Lys	ccc Pro	ggt Gly	aca Thr	aca Thr 235	gct Ala	tcc Ser	gat Asp	aac Asn	gtt Val 240	720
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caa Gln	gct Ala	gac Asp	aag Lys 340	Phe	gaa Glu	acc Thr	gtt Val	aca Thr 345	Ser	ggc Gly	aca Thr	aat Asn	gta Val 350	Thr	ttt Phe	1056
	agt Ser		Lys					Thr					Asp			1104
		Thr					Val					Ala			gtc Val	1152
															gca Ala	1200

xiii'

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•	385					390					395	<b>)</b>				400		
										aat Asn							1	248
	GIA	Ser	Ser	GIY	405	1.77		301	<b>(11)</b>	410	1)			Jer	415			
										gcc							1	296
•	Lys	Met	Asp	G1u 420	Thr		Asn			Ala					GIU	.; <u>(</u>		
	acc	cgc	aac	ggt	aaa	aat	atc	gac	atc	gcc	act	tcg	atg	gċgʻ	ccg	cag	1	344
	Thr	Arg	Asn 435	Gly	Lys	Asn	Ile	Asp 440	Ile	Ala	Thr	Ser	Met 445	Ala	Pro	Gln)	<i>:</i> ,	
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	Phe	Ser 450	Ser	Val	Ser	Leu	Gly 455	Ala	Gly	Ala	Asp	Ala 460	Pro	Thr	Leu	Ser		
	ata		gac	gag	aac	aca	tta	aat	atc	ggc	адс	aag	gat	acc	aac	aaa	. 1	440 .
	Val 465	Asp	Asp	Glu	Gly	Ala 470	Leu	Asn	Val	Gly	Ser 475	Lys	Asp	Thr	Asn	Lys 480		
·		at a		ξ./ <b>stt</b>	.; acc		at c	<b></b>	CCG	ggc		222	gan,	ada	gat	att)	4	488
	Pro	Val	Arg	Ile	Thr	Asn	Val	Ala	Pro	Gly 490	Val	Lys	Glu	Gly	Asp 495	Val		
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. ·	Thr	aac Asn	Val	Ala	Gln	Leu	Lys	Gly	Val	gcg Ala	Gln	Asn	Leu	Asn	Asn	Arg		
				500			•		505					510				504
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		٠.	515					520				•	525					
	gca Ala	acc Thr	gca Ala	ggt Gly	cta Leu	gtt <b>V</b> al	cag Gln	gcg Ala	tat Tyr	ctg Leu	Pro	ggc Gly	aag Lys	agt Ser	Met	atg Met	1	632
		530					-535	٠	1.		· ·	540						·
.,										gaa Glu								L <b>680</b>
	545					550			· · · · · · · · · · · · · · · · · · ·	Jan.	555	•				560	. (	
										tgg Trp								L728
	1	:		. :	565		• -			570			: 70 11		575			
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	Met 1		Lys	Ile	Tyr 5		Ile	Ile		Asn 10		Ala	Leu	Asn	Ala 15			
	Val	Val	. Val	Ser	Glu	Leu	Thr	Arq	Asn	His	Thr	Lys	Arg	Ala	Ser	Ala	•	•
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.7	Thr	Val	Ala 35	Thr	Ala	Val	Leu	Ala 40	Thr	Leu	Leu	Phe	Ala 45	Thr	Val	Gln
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	Lys	Ile	Lys 115	Gln	Asn	Thr	Asn	Glu 120	Asn	Thr	Asn	Asp	Ser 125	Ser	Phe	Thr
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	Lys 145	Leu	Ser	Phe	Gly	Ala 150	Asn	Gly	Asn	Lys	Val 155	Asn	Ile	Thr	Ser	Asp 160
	Thr	Lys	Gly	Leu	Asn 165	Phe	Ala	Lys	Glu	Thr 170		Gly	Thr	Asn	Gly 175	Asp
			Val	180					185					190	•	
	٠.		Thr 195		٠.			200			•		205			
	.*	210	•				215					220				
1	Trp 225		Ile			Val 230	Lys	Pro	Gly	Thr	Thr 235	Ala	Ser	Asp	Asn	Val 240
	 		Val		245	-				250					255	
1	• • •		Thr	260					265					270		
		•	Lys 275					280					285			
;	•	290			:		295	•				300				
	305		Glu		٠.	310					315				-	320
			Gly		325					330	1				335	
			Asp	340	1				345		٠			350		
	Ala	Ser	Gly 355	_	Gly	Thr	Thr	Ala 360		Val	Ser	Lys	Asp 365		Gln	Gly

Asn	Ile 370	Thr	Val	Lys	Tyr	<b>Asp</b> 375	Val	Asn	Val	Gly	Asp 380	Ala	Leu	Asn	Val	
Asn 385	Gln	Leu	Gln	Asn	Ser 390	Gly	Trp	Asn.	Leu	Asp 395	Ser	Lys	Ala	Val	Ala 400	
Gly	Ser	Ser	Gly	Lys 405	Val	Ile	Ser	Gly	Asn 410	Val	Ser.	Pro	Ser	Lys 415	Gly	
Lys	Met	Asp	Glu 420	Thr	Val	Asn	Ile	Asn 425	Ala	Gly	Asn	Asn	Ile 430	Glu	Ile	
Thr	Arg	Asn 435	Gly	Lys	Asn	Ile	Asp 440	Ile	Ala	Thr	Ser	Met 445	Ala	Pro	Gln	
Phe	Ser 450	Ser	Val	Ser	Leu	Gly 455	Ala	Gly	Ala	Asp	Ala 460	Pro	Thr	Leu	Ser	
Val 465	Asp	Asp	Glu	Gly	Ala 470	Leu	Asn	Val		Ser 475	Lys	Asp	Thr	Asn	Lys 480	
Pro	Val	Arg	Ile	Thr 485	Asn	Val	Ala	Pro	Gly 490	Val	Lys	Glu	Gly	Asp 495		,
Thr	Asn	Val	Ala 500	Gln	Leu	Lys	Gly	Val 505	Ala	Gln	Asn	Leu	Asn 510	Asn	Arg	
Ile	Asp	Asn 515	Val	Asp	Gly	Asn	Ala 520	Arg	Ala	Gly	Ile	Ala 525		Ala	Ile	
Ala	Thr 530	Ala	Gly	Leu	Val	Gln 535	Ala	Tyr	Leu	Pro	Gly 540		Ser	Met	Met	
Ala 545	Ile	Gly	Gly	Asp	Thr 550		Arg	Gly		Ala 555		Tyr	Ala	Ile	Gly 560	•
Tyr	Ser	Ser	Ile	Ser 565	Азр	Gly	Gly	Asn	Trp 570		Ile	Lys	Gly	Thr 575		
Ser	Gly	Asn	Ser 580	Arg	Gly	His	Phe	Gly 585		Ser	Ala	Ser	Val 590	Gly	Tyr	
Gln	Trp															
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	1> C		(178	5)												
	0> 8															40
atg Met 1	Asn	aaa Lys	ata Ile	tac Tyr 5	Arg	Ile	: att	tgg Trp	aat Asn 10	Ser	gcc Ala	Leu	aat Asn	gcc Ala 15	tgg Trp	48
gtc Val	gcc Ala	gta Val	tcc Ser 20	Glu	cto Leu	aca Thr	cgc Arg	aac Asn 25	His	acc Thr	aaa Lys	cgc Arg	geo Ala 30	Ser	gca Ala	96
acc	gtg	gcg	acc	gcc	gta	ttg	gcg	aca	ctg	ttg	ttt	gca	acq	gtt	cag	14

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		35					40				•	45		1		$\dot{\psi}$
gcg Ala	agt Ser 50	act Thr	acc Thr	gat Asp	gac Asp	gac Asp 55	gat Asp	tta Leu	tat Tyr	tta Leu	gaa Glu 60	ccc Pro	gta Val	caa Gln	cgc Arg	192
act Thr 65	gct Ala	gtc Val	gtg Val	ttg Leu	agc Ser 70	ttc Phe	cgt Arg	tcc Ser	gat Asp	aaa Lys 75	gaa Glu	ggc Gly	acg Thr	gga Gly	gaa Glu 80	240
aaa Lys	gaa Glu	gtt Val	aca Thr	gaa Glu 85	gat Asp	tca Ser	aat Asn	tgg Trp	gga Gly 90	gta Val	tat Tyr	ttc Phe	gac Asp	aag Lys 95	aaa Lys	288
gga Gly	gta Val	cta Leu	aca Thr 100	gcc Ala	gga Gly	aca Thr	atc Ile	acc Thr 105	ctc Leu	aaa Lys	gcc Ala	ggc Gly	gac Asp 110	aac Asn	ctg Leu	336
aaa Lys	atc Ile	aaa Lys 115	caa Gln	aac Asn	acc Thr	Asn	gaa Glu 120	aac Asn	acc Thr	aat Asn	gcc Ala	agt Ser 125	agc Ser	ttc Phe	acc Thr	384
tac Tyr	tcg Ser 130	ctg Leu	aaa Lys	aaa Lys	gac Asp	ctc Leu 135	aca Thr	gat Asp	ctg Leu	acc Thr	agt Ser 140	gtt Val	gga Gly	act Thr	gaa Glu	432
 aaa Lys 145	tta Leu	tcg Ser	ttt Phe	agc Ser	gca Ala 150	aac Asn	agc Ser	aat Asn	Lys	gtc Val 155	aac Asn	atc Ile	aca Thr	agc Ser	gac Asp 160	480
acc Thr	aaa Lys	ggc Gly	ttg Leu	aat Asn 165	ttc Phe	gcg Ala	aaa Lys	aaa Lys	acg Thr 170	gct Ala	gag Glu	acc Thr	aac Asn	ggc Gly 175	gac Asp	528
acc Thr	acg Thr	gtt Val	cat His 180	Leu	aac Asn	ggt Gly	atc Ile	ggt Gly 185	Ser	act Thr	ttg Leu	acc Thr	gat Asp 190	acg Thr	ctg Leu	576
ctg Leu	aat Asn	acc Thr 195	Gly	gcg Ala	acc Thr	aca Thr	aac Asn 200	Val	acc Thr	aac Asn	gac Asp	aac Asn 205	Val	acc	gat Asp	624 · ;
gac Asp	gag Glu 210	Lys	aaa Lys	cgt Arg	gcg	gca Ala 215	agc Ser	gtt Val	aaa Lys	gac Asp	gta Val 220	tta Leu	aac Asn	gca Ala	Gly	672
tgg Trp 225	Asn	att Ile	aaa Lys	ggc Gly	gtt Val 230	Lys	Pro	ggt Gly	aca Thr	aca Thr 235	gct Ala	tcc Ser	gat Asp	aac Asn	gtt Val 240	720
gat Asp	ttc Phe	gto Val	cgc Arg	act Thr 245	Tyr	gac Asp	aca Thr	gto Val	gag Glu 250	Phe	ttg Leu	agc Ser	gca Ala	gat Asp 255	Thr	768
aaa Lys	aca Thr	acg Thr	act Thr 260	: Val	aat Asn	gtg Val	gaa Glu	ago Ser 265	: Lys	gac Asp	aac Asn	ggc	aag Lys 270	Arg	acc Thr	816
gaa Glu	gtt Val	Lys 275	: Ile	ggt Gly	gcg Ala	aag Lys	act Thr 280	: Sei	gtt Val	ato Ile	aaa Lys	gaa Glu 285	Lys	gac Asp	ggt	864
aag Lys	tto Lev 290	ı Val	act l Thr	ggt Gly	aaa Lys	gac Asp 295	Lys	ggo Gly	gag Glu	g aat 1 Asr	gat Asp 300	Ser	tct Ser	aca Thr	gac Asp	912

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aaa Lys 305	ggc Gly	gaa Glu	ggc Gly	Leu	gtg Val 310	act Thr	gca Ala	aaa Lys	gaa Glu	gtg Val 315	att Ile	gat Asp	gca Ala	gta Val	aac Asn 320	960
														aca Thr 335		1008
				ttt										acc Thr		1056
														caa Gln		1104
														aac Asn		1152
aat Asn 385	cag Gln	ctg Leu	caa Gln	aac Asn	agc Ser 390	ggt Gly	tgg Trp	aat Asn	ttg Leu	gat Asp 395	tcc Ser	aaa Lys	gcg Ala	gtt Val	gca Ala 400	1200
ggt Gly	tct Ser	tcg Ser	ggc Gly	aaa Lys 405	gtc Val	atc Ile	agc Ser	ggc Gly	aat Asn 410	gtt Val	tcg Ser	ccg Pro	agc Ser	aag Lys 415	gga Gly	1248
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acc Thr	cgc Arg	aac Asn 435	ggc Gly	aaa Lys	aat Asn	atc Ile	gac Asp 440	atc Ile	gcc Ala	ạct Thr	tcg Ser	atg Met 445	acc Thr	ccg Pro	caa Gln	1344
Phe	tcc Ser 450	agc Ser	gtt Val	tcg Ser	ctc Leu	ggc Gly 455	gcg Ala	ggg	gcg Ala	gat Asp	gcg Ala 460	ccc Pro	act Thr	tta Leu	agc Ser	1392
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														gat Asp 495	Val	1488
				Gln					Ala					Asn	cac	1536
								Arg					Gln		att	1584
gca Ala	acc Thr 530	gca Ala	ggt Gly	ctg Leu	gtt Val	cag Gln 535	Ala	tat Tyr	ctg Leu	ccc Pro	ggc Gly 540	Lys	agt Ser	atg Met	atg Met	1632
gcg Ala 545	Ile	ggc Gly	ggc Gly	ggc	act Thr 550	Tyr	cgc Arg	ggc	gaa Glu	gcc Ala 555	Gly	tat Tyr	gcc Ala	ato Ile	ggc Gly 560	1680

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tac	tca	agc	att	tcc	gac	ggc	gga	aat	tgg	att	atc	aaa	ggc	acg	gcť 🖔	17
Tyr	Ser	Ser	Ile	Ser 565	Asp	Gly	Gly	Asn	Trp 570	Ile	Ile	Lys	Gly	Thr 575	Ala	1.
too.	aac	aat	tca	cac	aac.	cat	1000 ttc	aat	act	tee	gca.	tct	atc	gat	tat	17
Ser	Gly	Asn	Ser	Arg	Gly	His	Phe	Gly	Ala	Ser	Ala	Ser	Val	Gly	Tyr	7
:			580		· .	je .		585					590	.14 11λ		
-	tgg Trp						٠,		147 d	<b>.</b> .			, i		1.0	17
GIII.	Пр	595				. : •		<b>:</b>		.·· .		epin .		7		
									4							
	0> 9 1> 59	94						;	: · ·	· ' :	37			(8.)	137	•
<212	2> P	RT							• •					:		
<21.	3> N	eiss	erıa	men:	ıngı	C101:	3									
	0> 9 Asn	Lys	Ile	Tvr	Ara	Ile	 Ile	Tro	Asn	Ser	Ala	Leu	Asn	Ala	Trp	
1		-7,5		5	و ،		37		10					/ 15		::
Val	Ala	Val	Ser	Glu	Leu	Thr	Arg			Thr	Lys	Arg	Ala	Ser	Ala	N, A
			20					, 25		•.1	· ·	VO.	30	A A		;
Thr	Val	Ala	Thr	Ala	Val	Leu	Ala 40		Leu	Leu	Phe	Ala 45	Thr	Val	Gln	
·		35	·.·							1 / 1 1		1,000		97 34.		
Ala	Ser 50	Thr	Thr	Asp		Asp 55		Leu	Tyr	Leu	Glu 60		Val	Gln	Arg	• •
Thr	,	Val	Val	Ten	Ser	Phe	Ara	Ser	Asp	Lvs	Glu	Glv	Thr	Glv	Glu :	
65	n.a	Val	Val	Бец	70		9	,		75	<b>5_</b>			,02,	80	
Lys	Glu	Val	Thr	Glu	Asp	Ser	Asn	Trp	Gly	Val	Tyr	Phe	Asp			
		<i>:</i> .		85		,			90	•				95		
Gly	Val	Leu	Thr 100		Gly	Thr	Ile	Thr 105		Lys	Ala	Gly	Asp 110	Asn	Leu	· .
			·.		·;	. <u></u>			32.50	· (,		n <u>i</u> is				٠ ج د .
Lys	Ile	Lys 115		Asn	Thr	Asn	Glu 120		Thr	Asn	Ala	Ser 125		Phe	Thr	
Tur	Ser	Len	Lvs	t.vs	Aso	Leu	Thr	Asp	Leu	Thr	Ser	Val	Glv	Thr	Glu	
-1-	130			2,0		135		) <u>-</u>			140			1000 1000 1000		
Lys	Leu	Ser	Phe	Ser	Ala	Asn	Ser	Asn	Lys			Ile	Thr	Ser	Asp	
145					150					155				1	160	
Thr	Lys	Gly	Leu	Asn 165		Ala	Lys	Lys	Thr 170	Ala	Glu	Thr	Asn	Gly 175	Asp	
							<b>:.</b> :									
Thr	Thr	· Val	His 180		Asn	Gly		: Gly . 185			Leu	Thr	: Asp 190	Thr	Leu	
Len	) Den	ነ ሞኮ~	. G1	- 21≘	Thr	ሞh •	Δer	: Val	Thr	Aen	Agr	Acn	Val	Thr	Asp	
neu	. ASI	195		MIG			200				*	205		v :		
Asp	Glu	ı Lys	Lys	Arg	Ala	Ala	Ser	. Val	Lys	Asp		Leu	Asn	Ala	Gly	•
•	210		-	_		215					220		•	`.`		
-		ı Ile	Lys				Pro	Gly	Thr			Ser	Asp	Asn	Val	: .
225	)				. 230	)	٠.	•	٠.	235	٠. ز				240	

Asp Phe Val Arg Thr Tyr Asp Thr Val Glu Phe Leu Ser Ala Asp Thr

245		250 .	255
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<i>``</i>			•	٠.	245	•				250					255	
••	Lys	Thr		Thr 260	Val	Asn	Val	Glu	Ser 265	Lys	Asp	Asn	Gly	Lys 270	Arg	Thr
	Glu	Val	Lys 275	Ile	Gly	Ala	Lys	Thr 280	Ser	Val	Ile	Lys	Glu 285	Lys	Asp	Gly
· ·	Lys	Leu 290	Val	Thr	Gly	Lys	Asp 295	Lys	Gly	Glu	Asn	Asp 300	Ser	Ser	Thr	Asp
	Lys 305	Gly	Glu	Gly	Leu	Val 310	Thr	Ala	Lys	Glu	Val 315	Ile	Азр	Ala	Val	Asn 320
	Lys	Ala	Gly	Trp	Arg 325	Met	Lys	Thr	Thr	Thr 330	Ala	Asn	Gly	Gln	Thr 335	Gly
	Gln	Ala	Asp	Lys 340	Phe	Glu	Thr	Val	Thr 345	Ser	Gly	Thr	Asn	Val 350	Thr	Phe
) 11 13	Ala	Ser	Gly 355	Lys	Gly	Thr	Thr	Ala 360	Thr	Val	Ser	Lys	Asp 365	Asp	Gln	Gly
	Asn	Ile 370		Val	Met	Tyr	Asp 375		Asn	Val	Gly	Asp 380	Ala	Leu	Asn	Val
· (	Asn 385	Gln	Leu	Gln	Asn	Ser 390	Gly	Trp	neA	Leu	Asp 395	Ser	Lys	Ala	Val	Ala 400
	Gly	Ser	Ser	Gly	Lys 405	Val	Île	Ser	Gly	Asn 410	Val	Ser	Pro	Ser	Lys 415	Gly
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· 7	Phe	Ser 450		Val	Ser	Leu	Gly 455		Gly	Ala	Asp	Ala 460		Thr	Leu	Ser
	Val 465		Asp	Glu	Gly	Ala 470	Leu	Asn	Val	Gly	Ser 475		Asp	Ala	Asn	Lys 480
er' Historia National	Pro	Val	Arg		Thr 485		Val	Ala	Pro	Gly 490		Lys	Glu	Gly	Asp 495	Val
	Thr	Asn	Val	Ala 500		Leu	Lys	Gly		Ala	Gln	Asn	Leu	Asn 510		His
	Ile	Asp	Asn 515		Asp	Gly	Asn	Ala 520		Ala	Gly	Ile	Ala 525	Gln	Ala	Ile
	Ala	Thr 530		Gly	Leu	Val	Glr 535		Tyr	Leu	Pro	Gly 540		Ser	Met	Met
	Ala 545		Gly	Gly	Gly	Thr 550		Arg	Gly	Glu	Ala 555	Gly	Туг	Ala	Ile	Gly 560
;	Týr	Ser	Ser	Ile	Ser 565		Gly	/ Gly	Asn	570	Ile	: Ile	Lys	Gly	Thr 575	Ala

Ser Gly Asn Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr

580

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> Substitute Sheet (Rule 26) RO/AU

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aaa Lys	aaa Lys 210	cgt Arg	gcg Ala	gca Ala	agc Ser	gtt Val 215	aaa Lys	gac Asp	gta Val	tta Leu	aac Asn 220	gct Ala	ggc Gly	tgg Trp	aac Asn	672
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gtc Val	cgc Arg	act Thr	tac Tyr	gac Asp 245	aca Thr	gtc Val	gag Glu	ttc Phe	ttg Leu 250	agc Ser	gca Ala	gat Asp	acg Thr	aaa Lys 255	aca Thr	768
acg Thr	act Thr	gtt Val	aat Asn 260	gtg Val	gaa Glu	agc Ser	aaa Lys	gac Asp 265	aac Asn	ggc Gly	aag Lys	aaa Lys	acc Thr 270	gaa Glu	gtt Val	816
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gaa Glu 305	ggc Gly	tta Leu	gtg Val	act Thr	gca Ala 310	aaa Lys	gaa Glu	gtg <b>V</b> al	att Ile	gat Asp 315	gca Ala	gta Val	aac Asn	aag Lys	gct Ala 320	960
ggt Gly	tgg Trp	aga Arg	atg Met	aaa Lys 325	aca Thr	aca Thr	acc Thr	gct. Ala	aat Asn 330	ggt Gly	caa Gln	aca Thr	ggt Gly	caa Gln 335	gct Ala	1008
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tcg Ser	ggc	aaa Lys	gtc Val	Ile 405	Ser	ggc Gly	aat Asn	gtt Val	tcg Ser 410	ccg Pro	agc Ser	aag Lys	Gly	aag Lys 415	atg Met	1248
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aac Asn	ggt	aaa Lys 435	Asn	atc Ile	gac	atc Ile	gcc Ala 440	Thr	tcg Ser	atg Met	acc Thr	ccg Pro 445	cag Gln	ttt Phe	tcc Ser	1344
ago Ser	gtt Val 450	Ser	cto Leu	ggc Gly	gcg Ala	ggg Gly 455	Ala	gat Asp	gcg Ala	ccc Pro	act Thr 460	Leu	agc Ser	gtg Val	gat Asp	1392
ggg	gac	gca	ttg	aat	gto	ggc	ago	aag	aag	gac	aac	aaa	ccc	gtc	cgc	1440

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Gly 465	qeA	Ala	Leu		Val 470	Gly	Ser	Lys	Lys	Asp 475	Asn	Lys	Pro	Val	Arg 480	
			gtc Val													1488
			aaa Lys 500													1536
gtg Val	gac Asp	ggc Gly 515	aac Asn	gcg Ala	cgt Arg	gcg Ala	ggc Gly 520	atc Ile	gcc Ala	caa Gln	gcg Ala	att Ile 525	gca Ala	acc Thr	gca Ala	1584
ggt Gly	ctg Leu 530	gtt Val	cag Gln	gcg Ala	tat Tyr	ttg Leu 535	ccc Pro	ggc Gly	aag Lys	agt Ser	atg Met 540	atg Met	gcg Ala	atc Ile	ggc Gly	1632
ggc Gly 545	ggc Gly	act Thr	tat Tyr	cgc Arg	ggc Gly 550	gaa Glu	gcc Ala	ggt Gly	tac Tyr	gcc Ala 555	atc Ile	ggc Gly	tac Tyr	tcc Ser	agt Ser 560	1680
att Ile	tcc Ser	gac Asp	ggc Gly	gga Gly 565	aat Asn	tgg Trp	att Ile	atc Ile	aaa Lys 570	ggc Gly	acg Thr	gct Ala	tcc Ser	ggc Gly 575	aat Asn	1728
tcg Ser	cgc Arg	ggc Gly	cat His 580	ttc Phe	ggt Gly	gct Ala	tcc Ser	gca Ala 585	tct Ser	gtc Val	ggt Gly	tat T <u>y</u> r	cag Gln 590	tgg Trp	taa	1776
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<21: <21: <21: <400 Met	11> 59 22> P1 33> No 00> 1 Asn Val Val Ser 50	P1 RT P2 P3 P3 P3 P3 P3 P3 P3 P3 P3 P3 P3 P3 P3	Ser 20 Thr	Leu 5 Glu Ala Asn Val	Arg Leu Val Glu Ala 70 Lys	Thr Leu Glu 55	Ile Arg Ala 40 Gln Leu	Asn 25 Thr Glu	10 His Leu Glu Val	Thr Leu Asp Asn 75 Ser	Lys Phe Leu 60 Ser	Arg Ala 45 Tyr Asp	Ala 30 Thr Leu	Ser Val Asp	Ala Gln Pro Gly 80 Tyr	
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<21: <21: <400 Met 1 Val Thr Ala Val 65 Thr	1> 52 2> PP 3> No 0> 1. Asn Val Ser 50 Leu	Picker of the control	Ile Ser 20 Thr Asn Thr Lys 100	Leu 5 Glu Ala Asn Val Glu 85	Arg Leu Val Glu Ala 70 Lys	Thr Leu Glu 55 Val Val	Ile Arg Alaa40 Gln Leu Glu	Asn 25 Thr Glu Ile Glu Alaa 105	His Leu Glu Val Asn 90 Arg	Thr Leu Asp Asn 75 Ser	Lys Phe Leu 60 Ser Asp	Arg Ala 45 Tyr Asp	Ala 30 Thr Leu Lys Ala Leu 110	Ser Val Asp Glu Val 95 Lys	Ala Gln Pro Gly 80 Tyr	

#### xxiii

													•	•	
Ser 145	Phe	Ser	Ala	Asn	Gly 150	Asn	Lys	Val	Asn	Ile 155	Thr	Ser	qeA		Lys 160
Gly	Leu	Asn	Phe	Ala 165	Lys	Glu	Thr	Ala	Gly 170	Thr	Asn	Gly	Asp	Thr 175	Thr
Val	His	Leu	Asn 180	Gly	Ile	Gly	Ser	Thr 185		Thr	Asp	Thr	Leu 190	Leu	Asn
Thr	Gly	Ala 195	Thr	Thr	Asn	Val	Thr 200	Asn	qeA	neA	Val	Thr 205	Asp	Азр	Glu
Lys	Lys 210	Arg	Ala	Ala	Ser	Val 215	Lys	Asp	Val	Leu	Asn 220	Ala	Gly	Trp	Asn 
Ile 225	Lys	Gly	Val	Lys	Pro 230	Gly	Thr	Thr	Ala	Ser 235	Asp	Asn	Val	Asp	Phe 240
Val	Arg	Thr	Tyr	Asp 245	Thr	Val	Glu	Phe	Leu 250	Ser	Ala	Asp	Thr	Lys 255	Thr Thr
Thr	Thr	Val	Asn 260	Val	Glu	Ser	Lys '	Asp 265	Asn	Gly	Lys	Lys	Thr 270	Glu	Val
Lys	Ile	Gly 275	Ala	Lys	Thr	Ser	Val 280	Ile	Lys	Glu	Lys	Asp 285	Gly	Lys	Leu
Val	Thr 290	_	Lуз	Asp	Lys	Gly 295	Glu	Asn	Gly	Ser	Ser 300	Thr	Asp	Glu	Gly
G1u 305	Gly	Leu	Val	Thr	Ala 310	Lys	Glu	Val	Ile	Asp 315	Ala	Val	Asn	Lys	Ala 320
Gly	Trp	Arg	Met	Lys 325		Thr	Thr	Ala	Asn 330		Gln	Thr	Gly	Gln 335	Ala
Asp	Lys	Phe	Glu 340		Val	Thr	Ser	Gly 345	Thr	Asn	Val	Thr	Phe 350	Ala	Ser
Gly	Lys	Gly 355		Thr	Ala	Thr	Val 360	Ser	Lys	qeA	Asp	Gln 365		. Asn	Ile
Thr	Val 370		Tyr	Asp	Val	Asn 375		Gly	Asp	Ala	Leu 380		Val	Asn	Gln
Leu 385		Asn	Ser	Gly	Trp 390		Leu	qeA	Ser	Lys 395		Val	Ala	Gly	Ser 400
Ser	Gly	Lys	Val	. Ile 405		Gly	Asn	Val	Ser 410		Ser	Lys	Gly	415	
Asp	Glu	Thi	Val 420		ılle	. Asn	Ala	Gly 425		Asn	Ile	Glu	1le 430	Thr	Arg
Asr	Gly	Lys 435		ı Ile	e Asp		· Ala		Ser	Met	Thr	Pro 445		Phe	Ser
Ser	Va)		c Lev	ı Gly	y Ala	Gly 455		a Asp	Ala	Pro	Thr 460		Ser	. Val	Asp
Gl <sub>3</sub> 465		Ala	a Lev	ı Ası	n Val		y Sei	c Lys	Lys	475		Lys	Pro	Val	Arg 480
Ile	e Thi	r Ası	n Val	1 Ala		Gly	y Val	l Lys	Glu 490		, Asp	Va]	Thr	Asn 495	Val

#### xxiv

												•				
Ala	Gln	Leu	Lys 500	Gly	Val	Ala	Gln	<b>Asn</b> 505	Leu	Asn	Asn	Arg	Ile 510	Asp	Asn	•
Val	Asp	Gly 515	Asn	Ala	Arg	Ala	Gly 520	Ile	Ala	Gln	Ala	11e 525	Ala	Thr	Ala	• • •
Gly	Leu 530	Val	Gln	Ala	Tyr	Leu 535	Pro	Gly	Lys	Ser	Met 540	Met	Ala	Ile	Gly	
Gly 545	Gly	Thr	Tyr	Arg	Gly 550	Glu	Ala	Gly	Tyr	Ala 555	Ile	Gly	Tyr	Ser	Ser 560	
Ile	Ser	Asp	Gly	Gly 565	Asn	Trp	Ile	Ile	Lys 570	Gly	Thr	Ala	Ser	Gly 575	Asn	
Ser	Arg	Gly	His 580	Phe	Gly	Ala	Ser	Ala 585	Ser	Val	Gly	Tyr	Gln 590	Trp		
<211 <212	0> 12 1> 17 2> DN 3> Ne	197 NA	eria	meni	ingit	idis	3						₹ <del>!</del> .			
	0> 1> CI 2> (1		(179	7)												
<b>~40</b>	0> 12	,									·: :				-	
atg	aac Asn	aaa	ata Ile	tac Tyr 5	cgc Arg	atc Ile	att Ile	tgg Trp	aat Asn 10	agt Ser	gcc Ala	ctc Leu	aat Asn	gcc Ala 15	tgg Trp	48
gtc Val	gtc Val	gta Val	tcc Ser 20	gag Glu	ctc Leu	aca Thr	cgc Arg	aac Asn 25	cac His	acc	aaa Lys	cgc Arg	gcc Ala 30	tcc Ser	gca Ala	96
acc Thr	gtg Val	gcg Ala 35	acc Thr	gcc Ala	gta Val	ttg Leu	gcg Ala 40	Thr	ctg Leu	ttg Leu	ttt Phe	gca Ala 45	acg Thr	gtt Val	cag Gln	144
gcg Ala	aat Asn 50	Ala	acc Thr	gat Asp	gac Asp	gac Asp 55	Asp	tta Leu	tat Tyr	tta Leu	gaa Glu 60	Pro	gta Val	caa Gln	cgc Arg	192
act Thr 65	Ala	Val	Val	Leu	Ser	Phe	Arg	tcc Ser	Asp	Lys	Glu	gg <u>c</u> Gly	acg Thr	gga Gly	gaa Glu 80	240
aaa Lys	gaa Glu	ggt Gly	aca Thr	gaa Glu 85	Asp	tca Ser	aat Asn	tgg Trp	gca Ala 90	Val	tat Tyr	ttc Phe	gac Asp	gag Glu 95		288
aga Arg	gta JVal	cta Leu	aaa Lys 100	Ala	gga Gly	gca Ala	ato Ile	acc Thr 105	Leu	aaa Lys	geo Ala	ggc Gly	gac Asp 110	Asn	ctg Leu	336
aaa Lys	a ato	aaa Lys 115	Glr	aac Asr	acc Thr	aat Asr	gaa Glu 120	ı Asr	aco Thi	aat Asr	gaa Glu	aac Asr 125	Thr	aat Asn	gac Asp	384
agt Sei	t ago r Sei 130	Phe	aco Thi	tac Tyi	tcc Ser	cto Leu 135	ı Ly:	a aaa s Lys	a gad B Asp	cto Lev	aca Thi	: Asp	ctg Lev	acc Thr	agt Ser	432

											•					5.52
gtt Val 145	gaa Glu	act Thr	gaa Glu	aaa Lys	tta Leu 150	tcg Ser	ttt Phe	ggc Gly	gca Ala	aac Asn 155	ggt Gly	aat Asn	aaa Lys	gtc Val	aac Asn 160	480
atc Ile	aca Thr	agc Ser	gac Asp	acc Thr 165	aaa Lys	ggc Gly	ttg Leu	aat Asn	ttt Phe 170	gcg Ala	aaa Lys	gaa Glu	acg Thr	gct Ala 175	Gly ggg	528
acg Thr	aac Asn	ggc Gly	gac Asp 180	ccc Pro	acg Thr	gtt Val	cat His	ctg Leu 185	aac Asn	ggt Gly	atc Ile	ggt Gly	tcg Ser 190	act Thr	ttg Leu	576
								gcg Ala								624
aac Asn	gtt Val 210	acc Thr	gat Asp	gac Asp	gag Glu	aaa Lys 215	aaa Lys	cgt Arg	gcg Ala	gca Ala	agc Ser 220	gtt Val	aaa Lys	gac Asp	gta Val	<b>672</b>
tta Leu 225	aac Asn	gca Ala	ggc Gly	tgg Trp	aac Asn 230	att Ile	aaa Lys	ggc Gly	Val	Lys	ccc Pro	Gly	aca Thr	aca Thr	gct Ala 240	720
tcc Ser	gat Asp	aac Asn	gtt Val	gat Asp 245	ttc Phe	gtc Val	cgc Arg	act Thr	tac Tyr 250	gac Asp	aca Thr	gtc Val	gag Glu	ttc Phe 255	ttg Leu	768
agc Ser	gca Ala	gat Asp	acg Thr 260	aaa Lys	aca Thr	acg Thr	act Thr	gtt Val 265	aat Asn	gtg Val	gaa Glu	agc Ser	aaa Lys 270	gac Asp	aac Asn	816
ggc Gly	aag Lys	aaa Lys 275	acc Thr	gaa Glu	gtt Val	aaa Lys	atc Ile 280	ggt Gly	gcg Ala	aag Lys	act Thr	tct Ser 285	gtt Val	att Ile	aaa Lys	864
gaa Glu	aaa Lys 290	gac Asp	ggt Gly	aag Lys	ttg Leu	gtt Val 295	act Thr	ggt Gly	aaa Lys	Gly	aaa Lys 300	gac Asp	Glu	aat Asn	ggt Gly	912
tct Ser 305	tct Ser	aca Thr	gac Asp	gaa Glu	ggc Gly 310	gaa Glu	ggc Gly	tta Leu	gtg	act	gca	aaa Lys	gaa	gtg	att Ile 320	960
								aga Arg							Asn	1008
ggt Gly	caa Gln	aca Thr	ggt Gly 340	Gln	gct Ala	gac Asp	aag Lys	ttt Phe 345	Glu	acc Thr	gtt Val	aca Thr	tca Ser 350	Gly	aca Thr	1056
			Phe			Gly		Gly		Thr	Ala	Thr		Ser	aaa Lys	1104
gat Asp	gat Asp 370	Gln	ggc Gly	aac Asn	atc Ile	act Thr 375	Val	aag Lys	tat Tyr	gat Asp	gta Val 380	Asr	gtc Val	ggc Gly	gat Asp	1152
	Leu					Leu					Trp				tcc Ser 400	1200

•	aaa Lys	gcg Ala	gtt Val	gca Ala	ggt Gly 405	tct Ser	tcg Ser	ggc Gly	aaa Lys	gtc Val 410	atc Ile	agc Ser	ggc Gly	aat Asn	gtt Val 415	tcg Ser	1248
	ccg Pro	agc Ser	aag Lys	gga Gly 420	aag Lys	atg Met	gat Asp	gaa Glu	acc Thr 425	gtc Val	aac Asn	att Ile	aat Asn	gcc Ala 430	ggc Gly	aac Asn	1296
•	aac Asn	atc Ile	gag Glu 435	att Ile	acc Thr	cgc Arg	aac Asn	ggc Gly 440	aaa Lys	aat Asn	atc Ile	gac Asp	atc Ile 445	gcc Ala	act Thr	tcg Ser	1344
	atg Met	acc Thr 450	ccg Pro	caa Gln	ttt Phe	tcc Ser	agc Ser 455	gtt Val	tcg Ser	ctc Leu	ggc	gcg Ala 460	ggg Gly	gcg Ala	gat Asp	gcg Ala	1392
	ccc Pro 465	act Thr	tta Leu	agc Ser	gtg Val	gat Asp 470	gac Asp	gag Glu	ggc Gly	gcg Ala	ttg Leu 475	aat Asn	gtc Val	ggc Gly	agc Ser	aag Lys 480	1440
	gat Asp	gcc Ala	Asn	aaa Lys	ccc Pro 485	gtc Val	cgc Arg	att Ile	acc Thr	aat Asn 490	gtc Val	gcc Ala	ccg Pro	ggc Gly	gtt Val 495	aaa Lys	1488
	gag Glu	Gly ggg	gat Asp	gtt Val 500	aca Thr	aac Asn	gtc Val	gca Ala	caa Gln 505	ctt Leu	aaa Lys	ggt Gly	gtg Val	gcg Ala 510	caa Gln	aac Asn	1536
	ttg Leu	aac	aac Asn 515	cgc Arg	atc Ile	gac Asp	aat Asn	gtg Val 520	gac Asp	ggc Gly	aac Asn	gcg Ala	cgc Arg 525	gcg Ala	ggt Gly	atc Ile	1584
	gcc Ala	caá Gln 530	gcg Ala	att Ile	gca Ala	acc Thr	gca Ala 535	ggt Gly	ttg Leu	gct Ala	cag Gln	gcg Ala 540	tat Tyr	ttg Leu	ccc Pro	ggc Gly	1632
	aag Lys 545	agt Ser	atg Met	atg Met	gcg Ala	atc Ile 550	ggc	ggc Gly	ggt Gly	act Thr	tat Tyr 555	cgc Arg	ggc Gly	gaa Glu	gcc Ala	ggt Gly 560	1680
	tac Tyr	gcc Ala	atc Ile	ggc Gly	tac Tyr 565	tcg Ser	agc Ser	att Ile	tct Ser	gac Asp 570	act Thr	ggg Gly	aat Asn	tgg Trp	gtt Val 575	atc Ile	1728
	aag Lys	ggc Gly	acg Thr	gct Ala 580	Ser	ggc Gly	aat Asn	tcg Ser	cgc Arg 585	ggc	cat His	ttc Phe	ggt Gly	gct Ala 590	tcc Ser	gca Ala	1776
		Val		Tyr	cag Gln												1797
	<21 <21		98 RT	eria	men	ingi	tidi	. <b>s</b>									
	<40 Met		.3 Lys	Ile	Tyr 5		Ile	lle	Trp	Asn 10		Ala	Leu	Asn	Ala 15	Trp	

Substitute Sheet (Rule 26) RO/AU

20

Val Val Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala 25

30

## xxvii

Phr	Val	Ala 35	Thr	Ala	Val	Leu	Ala 40	Thr	Leu	Leu	Phe	Ala 45	Thr	Val	Gln
Ala	Asn 50	Ala	Thr	qeA	Asp	Asp 55	qeA	Leu	Tyr	Leų	Glu 60	Pro	Val	Gln	Arg
Thr 65	Ala	Val	Val	Leu	Ser 70	Phe	Arg	Ser	Asp	Lys 75	Glu	Gly	Thr	Gly	Glu 80
Lys	Glu	Gly	Thr	Glu 85	Asp	Ser	Asn	Trp	<b>Ala</b> 90	Val	Tyr	Phe	Asp	Glu 95	Lys
			100					Thr 105					110		
-		115					120	Asn				125			
	130					135		Lys			140				
145					150			Gly		155					160
				165				Asn	170					175	
			180					Leu 185					190		
		195					200	Ala				205			
	210					215		Arg			220				
225					230			Gly	•	235					240
	_			245				Thr Val	250					255	
			260					265 Gly					270		
		275					280					285			Gly
	290					295					300				
305	•				310					315					Ile 320
_				325					330	l				335	
			340	1				345					350	!	Thr
		355	<b>5</b>				360	)			•	365	•		Lys
Asp	Asp 370		Gly	Asn	ı Ile	Thr 375		Lys	Туг	- Asp	380		ı val	. СТУ	qeA v

## xxviii

					٠,				•					•			
	Ala 385	Leu	Asn	Val	Asn	Gln 390	Leu	Gln	Asn	Ser	Gly 395	Trp	Asn :	Leu i		Ser 400	• • •
1	Lys	Ala	Val	Ala	Gly 405	Ser	Ser	Gly	Lys	Val 410	Ile	Ser	Gly	Asn '	Val 415	Ser	•
1	?ro	Ser	Lys	Gly 420	Lys	Met	Asp	Glu	Thr 425	Val	Asn	Ile		Ala ( 430	Gly ,	Asn	
1	Asn	Ile	Glu 435	Ile	Thr	Arg	Asn	Gly 440	Lys	Asn	Ile	Asp	Ile . 445	Ala	Thr	Ser	
		450					455					Ala 460					• . •
•	465					470					475	Asn				480	
	_				485					490		Ala	÷		495		
				500					505			Gly		510			14.7
			515					520				Ala	525				
		530		•			535				:	Ala 540		•		•	·:
	545					550				•	555	Arg		•		560	
					565					570		Gly			575	•	
				580	_			Ser	Arg 585		His	Phe	GTÀ	590	ser	Ala	
	Ser	Val	. Gly 595	_	Gln	Trp	i						-	* *			
	<21 <21	0> 1 1> 1 2> 0	.800 NA	eria	nen	ingi	tidi	is		•	•		.1				
	<22 <22	:0> :1> C	CDS			-			<i>:</i>		•		• .			:	
	ato	Ası	c aaa	ata s Ile	a tac e Tyr	Arg	ato	c att	tgg Trp	aat Asr 10	Ser	gcc Ala	ctc Leu	aat Asn	gcc Ala 15	tgg Trp	48
	gto Val	gco L Ala	c gta a Vai	a tco L Sei 20	r Glu	g cto 1 Lei	aca 1 Th:	a cgo r Aro	aad y Asi 25	n His	acc Thr	aaa Lys	cgc Arg	gcc Ala 30	tcc Ser	gca Ala	96
٠,	acc Th	gte Va	g aad l Ly:	s Th	c gcd r Ala	gta Val	a tte	g gcq u Ala	a Th	g cto r Lei	g tto u Lei	ttt Phe	gca Ala 45	Thr	gtt Val	cag Gln	144

## xxix

gcg Ala	aat Asn 50	gct Ala	acc Thr	gat Asp	gaa Glu	gat Asp 55	gaa Glu	gaa Glu	gaa Glu	gag Glu	tta Leu 60	gaa Glu	ccc Pro	gta Val	gta Val	192
										gat Asp 75						240
										agt Ser						288
cac His	aac Asn	act Thr	cta Leu 100	cac His	ggc Gly	gca Ala	acc Thr	gtt Val 105	acc Thr	ctc Leu	aaa Lys	gcc Ala	ggc Gly 110	gac Asp	aac Asn	336
_							Asn 120			acc Thr				_		384
						tcg	ctg			gac Asp						432
_	-	-		_						gca Ala 155						480
										ttc Phe						528
ggg	acg Thr	aac Asn	ggc Gly 180	gac Asp	acc Thr	acg Thr	gtt Val	cat His 185	ctg Leu	aac Asn	ggt Gly	att Ile	ggt Gly 190	tcg Ser	act Thr	576
										acc Thr						624
							Lys			gcg Ala						672
	Leu					Asn				gtt Val 235						720
					Asp					tac Tyr						768
				Thr					Val	aat Asn						816
aac Asn	ggc Gly	aag Lys 275	Arg	acc Thr	gaa Glu	gtt Val	aaa Lys 280	Ile	ggt	gcg Ala	aag Lys	act Thr 285	tct Ser	gtt Val	att Ile	864
		Lys					Val			aaa Lys		Lys				912

		•											. ', '	:		: 12.
ggt Gly 305	tct Ser	tct Ser	aca Thr	gac Asp	gaa Glu 310	ggc Gly	gaa Glu	ggc Gly	tta Leu	gtg Val 315	act Thr	gca Ala	aaa Lys	gaa Glu	gtg Val 320	960
											aaa Lys					1008
											acc Thr					1056
aca Thr	aat Asn	gta Val 355	acc Thr	ttt Phe	gct Ala	agt Ser	ggt Gly 360	aaa Lys	ggt Gly	aca Thr	act Thr	gcg Ala 365	act Thr	gta Val	agt Ser	1104
											gat Asp 380					1152
gat Asp 385	gcc Ala	cta Leu	aac Asn	gtc Val	aat Asn 390	cag Gln	ctg Leu	caa Gln	aac Asn	agc Ser 395	ggt Gly	tgg Trp	aat Asn	ttg Leu	gat Asp 400	1200
tcc Ser	aaa Lys	gcg Ala	gtt Val	gca Ala 405	ggt Gly	tct Ser	tcg Ser	ggc Gly	aaa Lys 410	gtc Val	atc Ile	agc Ser	ggc Gly	aat Asn 415	gtt Val	1248
tcg Ser	ccg Pro	agc Ser	aag Lys 420	gga Gly	aag Lys	atg Met	gat Asp	gaa Glu 425	acc Thr	gtc Val	aac Asn	att Ile	aat Asn 430	gcc Ala	ggc Gly	1296
aac Asn	aac Asn	atc Ile 435	gag Glu	att Ile	acc Thr	cgc Arg	aac Asn 440	ggt Gly	aaa Lys	aat Asn	atc Ile	gac Asp 445	atc Ile	gcc Ala	act Thr	1344
tcg Ser	atg Met 450	acc Thr	ccg Pro	cag Gln	ttt Phe	tcc Ser 455	agc Ser	gtt Val	tcg Ser	ctc Leu	ggc Gly 460	gcg Ala	Gly	gcg Ala	gat Asp	1392
gcg Ala 465	Pro	act Thr	ttg Leu	agc Ser	gtg Val 470	Asp	gac Asp	aag Lys	ggc	gcg Ala 475	ttg Leu	aat Asn	gtc Val	ggc Gly	agc Ser 480	1440
aag Lys	gat Asp	gcc	aac Asn	aaa Lys 485	Pro	gtc Val	cgc Arg	att	acc Thr 490	Asn	gtc Val	gcc Ala	ccg Pro	ggc Gly 495	gtt Val	1488
aaa Lys	gag Glu	ggg	gat Asp 500	Val	aca Thr	aac Asn	gtc Val	gca Ala 505	Gln	ctt Leu	aaa Lys	ggc Gly	gtg Val 510	Ala	caa Gln	1536
			Asn					Val			aac Asn		Arg			1584
ato Ile	gcc Ala 530	Glr	gcg Ala	att Ile	gca Ala	acc Thr 535	Ala	ggt Gly	ctg Leu	gtt Val	cag Gln 540	Ala	tat Tyr	ctg Leu	ccc Pro	1632
ggc Gly 545	' Lys	agt Sei	ato Met	ato Met	g gcg : Ala 550	ılle	: ggc	ggc Gly	ggc Gly	act Thr 555	Tyr	cgc	ggc Gly	gaa Glu	gcc Ala 560	1680
ggt	tac	gco	ato	ggo	tac	tçc	: agt	att	tcc	gac	ggc	gga	aat	tgg:	att	1728

Gly Tyr Ala Ile Gly Tyr Ser Ser Ile Ser Asp Gly Gly Asn Trp 570 1 (0.45) 565 atc aaa ggc acg gct tcc ggc aat tcg cgc ggt cat ttc ggt gct tcc ' Ile Lys Gly Thr Ala Ser Gly Asn Ser Arg Gly His Phe Gly Ala Ser //j.585 (11) 580 gca tct gtc ggt tat cag tgg taa Ala Ser Val Gly Tyr Gln Trp <210> 15 <211> 599 <212> PRT <213> Neisseria meningitidis <400> 15 Met Asn Lys Ile Tyr Arg Ile Ile Trp Asn Ser Ala Leu Asn Ala Trp 45n 45n 10 Val Ala Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala Thr Val Lys Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln 45 Ala Asn Ala Thr Asp Glu Asp Glu Glu Glu Glu Leu Glu Pro Val Val 55 Arg Ser Ala Leu Val Leu Gln Phe Met Ile Asp Lys Glu Gly Asn Gly 70 Glu Asn Glu Ser Thr Gly Asn Ile Gly Trp Ser Ile Tyr Tyr Asp Asn 85 His Asn Thr Leu His Gly Ala Thr Val Thr Leu Lys Ala Gly Asp Asn 105 110 100 Leu Lys Ile Lys Gln Asn Thr Asn Lys Asn Thr Asn Glu Asn Thr Asn . 120 per 1996 - All (125 125 125 126) Asp Ser Ser Phe Thr Tyr Ser Leu Lys Lys Asp Leu Thr Asp Leu Thr 135 Ser Val Glu Thr Glu Lys Leu Ser Phe Gly Ala Asn Gly Asn Lys Val 155 145 Asn Ile Thr Ser Asp Thr Lys Gly Leu Asn Phe Ala Lys Glu Thr Ala 165 170 Gly Thr Asn Gly Asp Thr Thr Val His Leu Asn Gly Ile Gly Ser Thr 185 · 190 Leu Thr Asp Thr Leu Leu Asn Thr Gly Ala Thr Thr Asn Val Thr Asn 195 200 305 点: 为日

Substitute Sheet

250

235

220

255

Asp Asn Val Thr Asp Asp Lys Lys Lys Arg Ala Ala Ser Val Lys Asp

Val Leu Asn Ala Gly Trp Asn Ile Lys Gly Val Lys Pro Gly Thr Thr

Ala Ser Asp Asn Val Asp Phe Val His Thr Tyr Asp Thr Val Glu Phe

(Rule 26) RO/AU

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#### XXX11

					•				•					•		
	Leu	Ser	Ala	Asp 260	Thr	Lys	Thr	Thr	Thr 265	Val	Asn	Val	Glu	Ser 270	Lys	Asp
	Asn		Lys 275	Arg	Thr	Glu	Val	Lys 280	Ile	Gly	Ala	Lys	Thr 285	Ser	Val	Ile
		Glu 290		Азр	Gly	Lys	Leu 295	Val	Thr	Gly ·	Lys	Gly 300	Lys	Gly	Glu	Asn
	Gly 305	Ser	Ser	Thr	Asp	Glu 310	Gly	Glu	Gly	Leu	Val 315	Thr	Ala	Lys	Glu	Val 320
:				(	325	Ì	٠.			330				Thr	335	
				340					345					Thr 350		
	4 2		355			. :	•	360				• • •	365	• '		
•		370			'. 		375					380		Asn		-
	385	٠.			3.	390					395	,		Asn	Ţ,	400
					405 :				· : ·	410		•	. •	Gly	415	
				420		,			425					Asn 430		
			435	•			٠.	440					445			
		450			<i>:</i>	•	455	ı				460		Gly		
	465			.:		470					475			Val		480
	; 👬:	. ::	(1) (		485			•.		490	) : •		•	Pro	495	
		•	ia te si	500					505	5	•			510		Gln
	. ; / '		515	,				520	)	•			525	•		Gly
	٠,	530	)				535	5				540	)			Pro
	545		. •			550	)				555	5				560
	_		•		565	5				570	)				575	
	Ile	Ly:	s Gly	y Thi 580		s Sei	Gl	y Ası	n Se: 58:		g Gly	y His	s Phe	9 Gly	, Alâ )	ser
	Ala	a Se	r Va	1 G1	у Ту	r Glı	n Tr	p								

## xxxiii

595

				•
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gtc gcc gta tcc Val Ala Val Ser 20	gag ctc aca Glu Leu Thr	cgc aac cac Arg Asn His 25	acc aaa cgc o Thr Lys Arg A	gcc tcc gca 96 Ala Ser Ala 30
acc gtg aag acc Thr Val Lys Thr 35	gcc gta ttg Ala Val Leu	gcg aca ctg Ala Thr Leu 40	ttg ttt gca Leu Phe Ala 45	acg gtt cag 144 Thr Val Gln
gcg aat gct acc Ala Asn Ala Thr 50	gat gaa gat Asp Glu Asp 55	gaa gaa gaa Glu Glu Glu	gag tta gaa Glu Leu Glu : 60	tcc gta caa 192 Ser Val Gln
cgc tct gtc gta Arg Ser Val Val 65	ggg agc att Gly Ser Ile 70	caa gcc agt Gln Ala Ser	atg gaa ggc Met Glu Gly 75	agc gtc gaa 240 Ser Val Glu 80
ttg gaa acg ata Leu Glu Thr Ile	tca tta tca Ser Leu Ser 85	atg act aac Met Thr Asn 90	gac agc aag Asp Ser`Lys	gaa ttt gta 288 Glu Phe Val 95
gac cca tac ata Asp Pro Tyr Ile 100	Val Val Thr	Leu Lys Ala 105	Gly Asp Asn	Leu Lys Ile 110
aaa caa aac acc Lys Gln Asn Thr 115	aat gaa aac Asn Glu Asn	acc aat gcc Thr Asn Ala 120	Ser Ser Phe 125	acc tac tcg 384 Thr Tyr Ser
ctg aaa aaa gac Leu Lys Lys Asp 130	ctc aca ggc Leu Thr Gly 135	ctg atc aat Leu Ile Asn	gtt gaa act Val Glu Thr 140	gaa aaa tta 432
tcg ttt ggc gca Ser Phe Gly Ala 145	a aac ggc aag Asn Gly Lys 150	Lys Val Asn	atc ata agc Ile Ile Ser 155	gac acc aaa 480 Asp Thr Lys 160
ggc ttg aat ttc Gly Leu Asn Phe	c gcg aaa gaa e Ala Lys Glu .165	acg gct ggg Thr Ala Gly 170	Thr Asn Gly	gac acc acg 528 Asp Thr Thr 175
gtt cat ctg aad Val His Leu Ass 180	Gly Ile Gly	tcg act ttg Ser Thr Leu 185	Thr Asp Met	ctg ctg aat 576 Leu Leu Asn 190
acc gga gcg acc Thr Gly Ala Thr 195	c aca aac gta r Thr Asn Val	acc aac gac Thr Asn Asp 200	aac gtt acc Asn Val Thr 205	gat gac gag 624 Asp Asp Glu

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## xxxiv

		•														
											aac Asn 220					672
											gat Asp					720
											gca Ala					768
							Lys				aag Lys					816
											aaa Lys					864
gtt Val	act Thr 290	ggt Gly	Lys	ggc Gly	aaa Lys	ggc Gly 295	gag Glu	aat Asn	ggt Gly	tct Ser	tct Ser 300	aca Thr	gac Asp	gaa Glu	ggc Gly	912
											gca Ala					960
ggt Gly	tgg Trp	aga Arg	atg Met	aaa Lys 325	aca Thr	aca Thr	acc Thr	gct Ala	aat Asn 330	ggt Gly	caa Gln	aca Thr	ggt Gly	caa Gln 335	gct Ala	1008
		Phe									gta Val					1056
											gat Asp					1104
	Val 370										cta Leu 380					1152
ctg	caa Gln										gcg Ala					1200
											agc Ser				Met	1248
				Asn					Asn		atc Ile			Thr		1296
aac Asn	ggc	aaa Lys 435	Asn	atc	gac Asp	atc	gcc Ala 440	Thr	tcg Ser	atg Met	acc Thr	ccg Pro 445	Gln	ttt Phe	tcc	1344
ago Ser	gtt Val 450	Ser	cto Leu	ggc Gly	gcg Ala	ggg Gly 455	Ala	gat Asp	gcg Ala	Pro	act Thr 460	Leu	agc Ser	gtg Val	gat Asp	1392
gac	gag	ggc	gcg	ttg	aat	gto	ggc	ago	aag	gat	gcc	aac	aaa	ccc	gtc	1440

#### XXXV

Asp 465	Glu	Gly	Ala	Leu	Asn 470	Val	Gly	Ser	Lys	Asp 475	Ala	Aśn	Lys	Pro	Val 480	
cgc Arg																1488
gtc Val															gac Asp	1536
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Ala	ggt Gly 530	ctg Leu	gtt Val	cag Gln	gcg Ala	tat Tyr 535	ctg Leu	ccc Pro	ggc Gly	aag Lys	agt Ser 540	atg Met	atg Met	gcg Ala	atc Ile	1632
ggc Gly 545	ggc Gly	ggc Gly	act Thr	tat Tyr	ctc Leu 550	ggc Gly	gaa Glu	gcc Ala	ggt Gly	tat Tyr 555	gcc Ala	atc Ile	ggc Gly	tac Tyr	tca Ser 560	1680
agc Ser	att Ile	tcc Ser	gcc Ala	ggc Gly 565	gga Gly	aat Asn	tgg Trp	att Ile	atc Ile 570	aaa Lys	ggc Gly	acg Thr	gct Ala	tcc Ser 575	ggc Gly	1728
aat Asn	tcg Ser	cgc Arg	ggc Gly 580	cat His	ttc Phe	ggt Gly	gct Ala	tcc Ser 585	gca Ala	tct Ser	gtc Val	ggt Gly	tat Tyr 590	cag Gln	tgg Trp	1776
taa							-		• :				•			1779
taa <210	)> 1 <sup>'</sup>										. ; <sup>-</sup>		٠			1779
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Substitute Sheet (Rule 26) RO/AU

Lys Gln Asn Thr Asn Glu Asn Thr Asn Ala Ser Ser Phe Thr Tyr Ser 115 120 125

	•	•							41.50				61 13	1.53	<b>分别</b> 是
Leu	Lys 130	Lys	Asp	Leu .	Thr	Gly 135	Leu	Ile .	Asn		G1u 140	Thr	Glu	Lys	Leu
Ser 145	Phe	Gly	Ala	Asn	Gly 150	Lys	Lys	Val		Ile 155	Ile	Ser	Asp	Thr	Lys 160
Gly	Leu	Asn	Phe	Ala 165	Lys	Glu	Thr		Gly 170	Thr	Asn	Gly	Asp	Thr 175	Thr
Val	His	Leu	Asn 180	Gly	Ile	Gly		Thr 185	Leu	Thr	Asp	Met	Leu 190	Leu	Asn
Thr	Gly	Ala 195	Thr	Thr	Asn		Thr 200	Asn '	Asp	Asn	Val	Thr 205	Asp	Asp	Glu
Lуз	Lys 210	Arg	Ala	Ala	Ser	Val 215	Lys	Asp	Val	Leu	Asn 220	Ala	Gly	Trp	Asn
11e 225	Lys	Gly	Val	Lys	Pro 230	Gly	Thr	Thr	Ala	Ser 235	Asp	Asn	Val	Asp	Phe 240
Vıl	Arg	Thr	Tyr	Asp 245	Thr	Val	Glu	Phe	Leu 250	Ser	Ala	Asp	Thr	Lys 255	
Thr	Thr	Val	Asn 260	Val	Glu	Ser	Lys	Asp 265	Asn	Gly	Lys	Lys	Thr 270	Glu	Val
Lys	Ile	Gly 275	Ala	Lys	Thr	Ser	Val 280	Ile	Lys	Glu	Lys	Asp 285	Gly	Lys	Leu
Val	Thr 290	Gly	Lys	Gly	ГÀЗ	Gly 295	Glu	Asn	Gly	Ser	Ser 300	Thr	Asp	Glu	Gly
305	_	٠.			Ala 310					315	•				320
_	_	_		325	Thr				330			•		335	
-	,	,	340		Val		••/	345	(1), (			e generalis F	350		in and the
_	•	355		· ·	Ala	ight.	360	. :			, / .	365	1,410	Υb.,	$L^{2,N}(\mathbb{R})$
	370			•	Val	375	•."				380		42		٠., .
385	•				Trp 390					395			:	٠,٠,٠	400
				405				•	410		· ·	•	;	415	
_		•	420	)	Ile	•		425	• .*	•		,	430	13.2	•
		435	5		Asp		440			•	• •	445	,		
	450					455	•				460	)		•	Asp
As <sub>1</sub>		ı Gly	, Ala	a Lev	470		Gly	Ser	Lys	475	Ala	A ASI	Lys	Pro	Val 480

# .. xxxvii

	Arg	Ile	Thr	Asn	Val 485	Ala	Pro	Gly	Val	Lys 490	Glu	Gly	qeA	Val	Thr 495	Asn	
	Val	Ala	Gln	Leu 500		Gly	Val		G1n 505	Asn	Leu	Asn	Asn	Arg 510	Ile	Asp	
	Asn	Val	Asn 515	Gly		Ala	Arg	Ala 520		Ile	Ala	Gln	Ala 525	Ile	Ala	Thr	
•	Ala	Gly 530	Leu	Val	Gln	Ala	Tyr 535	Leu	Pro	Gly	Lys	Ser 540	Met	Met	Ala	Ile	*
	Gly 545	Gly	Gly	Thr	Tyr	Leu <sub>.</sub> 550	Gly	Glu	Ala	Gly	Tyr 555	Ala	Ile	Gly	Tyr	Ser 560	
	Ser	Ile	Ser	Ala	Gly 565	Gly	Asn	Trp	Ile	Ile 570	Lys	Gly	Thr	Ala	Ser 575	Gly	•
	Asn	Ser	Arg	Gly 580		Phe	Gly	Ala	Ser 585	Ala	Ser	Val	Gly	Tyr 590	Gln	Trp	
•	 <210	0> 1	B	) . Q		d	•	:	•	;‡							٠.
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٠,			,.		• ! • !		٠,٠		-						•		
	<220	-	D.C.						: :		<i>.</i> .						
		1> C 2> /		(177	0)												
	\22	27 (	1/	(1	,												•
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	Met 1		rys		Tyr 5		rie	ire	rrp	10	261	ита	Deu	HOII	15	ilp	
					•		•					•					
	gta	gtc	gta	tcc	gag	ctc	aca	cgc	aac	cac	acc	aaa	cgc	gcc	tcc	gca	96
	Val	Val	Val	Ser 20		Leu	Thr	Arg	Asn 25	HIS	Thr	гуз	Arg	30	Ser	WIG	
٠.	·.: .					Ł	•			-		٠					
. •	acc	gtg	gcg	acc	gcc	gta	ttg	gcg	aca	ctg	ctg	tcc	gca	acg	gtt	cag	144
	Thr	Val	Ala 35	Thr	Ala	Val	Leu	Ala 40		Leu	Leu	ser	A1a 45	Thr	Val	GIN	
			1.	C = Q			L =										
• •	gcg	aat	gct	acc	gat	acc	gat	gaa	gat	gaa	gag	tta	gaa	tcc	gta	gca	192
	Ala	Asn 50		Thr	Asp	Thr	Asp 55		Asp	GIU	GIU	Leu 60	GIU	Ser	vaı	ATS	
į		-			i												
	cgc	tct	gct	ctg	gtg	ttg	caa	ttc	atg	atc	gat	aaa	gaa	ggc	aat	gga	240
,	Arg 65		Ala	Leu	val	. ьеи 70		rne	Met	TIE	ASP 75		GIU	GIY	ASII	80	
				٠,	•	٠.											
	gaa	ato	gaa	tct	aca	gga	gat	ata	ggt	tgg	agt	ata	tat	tac	gac	gat	288
	Glu	ı İle	GIU	Ser	1'nr		Asp	) ite	GIA	90		rre	ıyı	ıyı	95	Asp	
			•		•												
	cac	aac	act	cta	cac	ggc	gca	acc	gtt	acc	ctc	aaa	gcc	ggc	gac	aac	336
	His	s Asr	Thi	Leu 100	•	. GT	ALE	rnr	vai 105		Leu	гъ	HIG	110		nsil	
			٠.	,	•		•										
	cto	g aaa	ato	aaa	caa	ago	ggo	aaa	gac	tto	acc	tac	tcg	ctg	aaa	aaa	384
	ctg Let	aaa 1 Lys	ato Ile 115	Lys	caa Glr	ago Ser	ggc Gly	aaa Lys 120	Asp	tto Phe	acc Thr	tac Tyr	tcg Ser 125	Leu	aaa Lys	aaa Lys	384

# xxxviii

gag Glu	ctg Leu 130	aaa Lys	gac Asp	ctg Leu	acc Thr	agt Ser 135	gtt Val	gaa Glu	act Thr	gaa Glu	aaa Lys 140	tta Leu	tcg Ser	ttt Phe	ggc Gly	432
gca Ala 145	aac Asn	ggt Gly	aat Asn	aaa Lys	gtc Val 150	aac Asn	atc Ile	aca Thr	agc Ser	gac Asp 155	acc Thr	aaa Lys	ggc Gly	ttg Leu	aat Asn 160	480
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tct Ser	cac His	gtt Val 195	gat Asp	gcg Ala	ggt Gly	aac Asn	caa Gln 200	agt Ser	aca Thr	cat His	tac Tyr	act Thr 205	cgt Arg	gca Ala	gca Ala	624
agt Ser	att Ile 210	aag Lys	gat Asp	gtg Val	ttg Leu	aat Asn 215	gcg Ala	ggt Gly	tgg Trp	aat Asn	att Ile 220	aag Lys	ggt Gly	gtt Val	aaa Lys	672
act Thr 225	ggc Gly	tca Ser	aca Thr	act Thr	ggt Gly 230	caa Gln	tca Ser	gaa Glu	aat Asn	gtc Val 235	gat Asp	ttc Phe	gtc Val	cgc Arg	act Thr 240	720
Tyr	Asp	Thr	gtc Val	G1u 245	Phe	Leu	Ser	Ala	Asp 250	Thr	Lys	Thr	Thr	Thr 255	Val	768
aat Asn	gtg Val	gaa Glu	agc Ser 260	aaa Lys	gac Asp	aac Asn	ggc Gly	aag Lys 265	aga Arg	acc Thr	gaa Glu	gtt Val	aaa Lys 270	atc Ile	ggt Gly	816
Ala	Lys	Thr 275		Val	Ile	Lys	Glu 280	Lys	Asp	Gly	Lys	Leu 285	Val	Thr	Gly	864
Lys	Gly 290	Lys	ggc Gly	Glu	Asn	Gly 295	Ser	Ser	Thr	Asp	Glu 300	Gly	Glu	Gly	Leu	912
Val 305	Thr	Ala	Lys	Glu	Val 310	Ile	Asp	Ala	Val	Asn 315	Lys	Ala	Gly	Trp	320	960
atg Met	aaa Lys	aca Thi	aca Thr	acc Thr 325	Ala	aat Asn	ggt	caa Gln	aca Thr 330	Gly	caa Gln	gct Ala	gac Asp	aag Lys 335	Phe	1008
gaa Glu	acc Thr	gtt Val	aca L Thr 340	Ser	ggc Gly	aca Thr	aaa Lys	gta Val 345	Thr	ttt Phe	gct Ala	agt Ser	ggt Gly 350	Asn	ggt	1056
aca Thi	a act	gc; Ala 35	a Thi	gta Val	agt LSer	aaa Lys	gat Asp 360	Asp	caa Gln	ggc	aac Asn	Ile 365	Thr	gtt Val	aag Lys	1104
tat Ty	gat Asp 370	va:	a aat 1 Asr	gto Val	ggc Gly	gat Asp 375	Ala	cta Lev	aac Asn	gto Val	aat Asn 380	Glr	ctg Lev	caa Glr	aac Asn	1152
age	c ggt	t tg	g aat	tt	g gat	tco	aaa	a gcc	gtt	gca	ggt	tct	tc	g ggc	aaa	1200

## xxxix

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Ser 385	Gly	Trp	Asn	Leu	Asp 390	•	Lys	Ala	Val	Ala 395	Gly	Ser	Ser	Gly	Lys 400	
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gtc Val	aac Asn	att Ile	aat Asn 420	gcc Ala	ggc Gly	aac Asn	aac Asn	atc Ile 425	gag Glu	att Ile	acc Thr	cgc Arg	aac Asn 430	ggc Gly	aaa Lys	1296
aat Asn	atc Ile	gac Asp 435	atc Ile	gcc Ala	act Thr	Ser	atg Met 440	acc Thr	ccg Pro	caa Gln	ttt Phe	tcc Ser 445	agc Ser	gtt Val	tcg Ser	1344
ctc Leu	ggc Gly 450	gcg Ala	G1y ggg	gcg Ala	gat Asp	gcg Ala 455	ccc Pro	act Thr	tta Leu	agc Ser	gtg Val 460	gat Asp	gac Asp	gag Glu	ggc Gly	1392
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Asn	Val	Ala	Pro	Gly 485	Val	Lys	Glu	Gly	Asp 490	Val	Thr	Asn	Val	gca Ala 495	Gln	1488
ctt Leu	aaa Lys	ggt Gly	gtg Val 500	gcg Ala	caa Gln	aac Asn	ttg Leu	aac Asn 505	aac Asn	cgc Arg	atc Ile	gac Asp	aat Asn 510	gtg Val	aac Asn	1536
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gct	cag Gln 530	gcc Ala	tat Tyr	ttg Leu	ccc Pro	ggc Gly 535	aag Lys	agt	atg Met	atg Met	gcg Ala 540	atc Ile	ggc	ggc	ggt Gly	1632
act Thr 545	tat Tyr	ctc Leu	ggc	gaa Glu	gcc Ala 550	Gly	tac Tyr	gcc Ala	atc Ile	ggc Gly 555	Tyr	tcg Ser	agc Ser	att Ile	tct Ser 560	1680
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<211> 589

<212> PRT

<213> Neisseria meningitidis

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Val Val Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala 20 25 30

Thr Val Ala Thr Ala Val Leu Ala Thr Leu Leu Ser Ala Thr Val Gln

							_		-						•
		35					40					45			·:
Ala	Asn 50	Ala	Thr	Asp	Thr	Asp 55	Glu	Asp	<b>Gl</b> u	Glu	Leu 60	G1u	Ser	Val	Ala
Arg 65	Ser	Ala	Leu	Val	Leu 70	Gln	Phe	Met	Ile	Asp 75	Lys	Glu	Gly	Asn	Gly 80
Glu	Ile	Glu	Ser	Thr 85	Gly	Asp	Ile	Gly	Trp 90	Ser	Ile	Tyr	Tyr	Asp 95	Asp
His	Asn	Thr	<b>Le</b> u 100	His	Gly	Ala	Thr	Val 105	Thr	Leu	Lys	Ala	Gly 110	Ąsp	Asn
Leu	Lys	Ile 115	Lys	Gln	Ser	Gly	Lys 120	Asp	Phe	Thr	Tyr	Ser 125	Leu	Lys	Lys
Glu	Leu 130	Lys	Asp	Leu	Thr	Ser 135	Val	Glu	Thr	Glu	Lys 140	Leu	Ser	Phe	Gly
Ala 145	Asn	Gly	Asn	Lys	Val 150	Asn	Ile	Thr	Ser	Asp 155	Thr	Lys	Gly	Leu	Asn 160
Phe	Ala	Lys	Glu	Thr 165	Ala	Gly	Thr	Asn	Gly 170	Asp	Pro	Thr	Val	His'	Leu
Asņ	Gly	Ile	Gly 180	Ser	Thr	Leu	Thr	Asp 185	Thr	Leu	Ala	Gly	Ser 190	Ser	Ala
Ser	His	Val 195	Asp	Ala	Gly	Asn	Gln 200	Ser	Thr	His	Tyr	Thr 205	Arg	Ala	Ala
Ser	Ile 210	Lys	Asp	Val	Leu	Asn 215	Ala	Gly	Trp	Asn	11e 220	Lys	Gly	Val	Lys
Thr 225	Gly	Ser	Thr	Thr	Gly 230	Gln	Şer	Glu	Asn	Val 235	Asp	Phe	Val	Arg	Thr 240
Tyr	Asp	Thr	Val	Glu 245		Leu	Ser	Ala	Asp 250		Lys	Thr	Thr	Thr 255	Val
Asn	Val	Glu	Ser 260		<b>Asp</b>	Asn	Gly	Lys 265		Thr	Glu	Val	Lys 270	Ile	Gly
Ala	Lys	Thr 275		Val	Ile	Lys	Glu 280		Asp	Gly	Lys	Leu 285	Val	Thr	Gly
Lys	Gly 290	_	Gly	Glu	Asn	Gly 295		Ser	Thr	Asp	Glu 300	Gly	Glu	Gly	Leu
Val 305		Ala	Lys	Glu	Val 310		Asp	Ala	Val	Asn 315		Ala	Gly	Trp	Arg 320
Met	Lys	Thr	Thr	Thr 325		Asn	Gly	Gln	330		Gln	Ala	Asp	Lys 335	Phe
Glu	Thr	· Val	Thr 340		Gly	Thr	Lys	Val 345		Phe	Ala	Ser	Gly 350		Gly
Thi	Thi	355		· Val	. Ser	Lys	360		Glr	Gly	Asn	11e 365		Val	Lys
Туз	Ası	Va]	l Asr	ı Val	Gly	/ Asp	Ala	Lev	ı Asr	val	. Asr	Glr	Leu	Gln	Asn

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	Sar	GI v	Trn	Δen	Leu .	Asn	Ser 1	.vs /	Ala '	Val .	Ala	Glv	Ser	Ser	Glv	Lvs	
	385	GIY	rrp	non		390		-,-			395		1	7,71		400	
	303					050	•		í ,							:	
	Ual	Tla	Sor	Glv	Asn	۷a۱	Ser 1	Pro !	Ser	ī.ve	ตาง ์	I.vs	Het.	Asp	Glu	Thr	
	Vai	116	Ser	GLY	405	141	<b>.</b>	337		410					415	V()	
					403		•	1,00	3	7617		46					
	**- 1	B	<b>T1</b> -	200	Ala	C111	Non I	nen '	רום	Glus	Tla	Thr.	Ara .	Δen	GI v	T.ve	, ,,
	vaı	ASN	TTE		Ald	GIY	MSII A		425	GIU.	TIE.	`		430	GLy	Lys .	٠.
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		450					455		•	1. (1)		460	· · ·.				
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	Glv	Agn	Ala	Ara	Ala	Glv	Ile	Ala	Gln	Ala	Ile	Ala	Thr	Ala	Gly	Leu	* * * *
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7	712	Gla	212	Tur	Leu	Pro	Glv	I.vs	Ser	Met	Met	Ala	Ile	Glv	Glv	Glv	\$ 1 1930
٠,	AIG	530	ALU	- 7 -	200		535	-1-		3.7		540					
		330			•		333							42.5			1300
	mb	m	T 011	C1	Glu	Δ12	Gly	ጥህታ	Δla	Tla	Glv	Tur	Ser	Ser	Tle	Ser	
		ıyı	Leu	GLY	Giu	550	GLY	1 Y L	nzu	110	555		-	-		560	
	545					330				X	333	·`	•			.005	
	_				Trp	Tto 1	т1 о	T	c1	mb ~		807	G1.	Non	Sor	Ara	
	Asp	Thr	GIY	ASII	565	Val	116	цуэ	GLY	570		361	.013		575		• • •
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		•••	n	G1	Thr	00=	712	602	17-1	Clar	Tur	G1n	Trans.				
•	GTÄ	HIS	Pne			ser	АТА	Ser.	585	GLY	LYL	GIII	TIP		٠.		•
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	Val	Va	L Va	L Ser	Glu	Leu	Thr	Ara	Asn	His	Thr	Lys	Ara	Ala	Ser	Ala	
	741			20				- 9	25		_			30		٠	
				٠.	-											•	•
			7 33	7 200	gcc	· //+ =	tt~	aca	act	Ctd	tta	+++	gca	aco	att	cao	144
			y ad	y acc	, yuu	. y.a	LLY	y Ly	Th~	Len	Tan	Phe	Ala	Thr	Val	Gln	
	acc	. yc.	7	<sub>-</sub> ጥኤ-	~ N1~	יפעו			1115	ຸມເປ	. ueu	* ****		***			
	acc Thi	· Va	L Ly		r Ala	val	. Leu										
	acc Thi	va.	1 Ly.		r Ala	val	. beu	40		• •	•		45				
	Thi	va.	1 Ly.	5				40				•	45				•
	Thi	va.	Ly. 3	5 taad	c aat	: gaa	gag	40 caa	gaa	gaa	gat	. tta	45 tat	tta	gac	ccc	192
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	Thi	va.	Ly. 3 t gc r Al	5 taad	c aat	: gaa	gag	40 caa Gln	gaa	gaa	gat	. tta	45 tat Tyr	tta	gac	ccc	192

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tto Phe	aac Asn	gag Glu	aaa Lys 100	gga Gly	gta Val	cta Leu	aca Thr	gcc Ala 105	aga Arg	gaa Glu	atc Ile	acc Thr	ctc Leu 110	aaa Lys	gcc Ala	336
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# xliii

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## xliv

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Ser Phe Ser Ala Asn Gly Asn Lys Val Asn Ile Thr Ser Asp Thr Lys 145 150 155 160

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Thr Thr Val Asn Val Glu Ser Lys Asp Asn Gly Lys Lys Thr Glu Val 260 265 270

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#### xlvi

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#### xlvii

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 98/01031

A.	CLASSIFICATION OF SUBJECT MATTER		
Int Cl <sup>6</sup> :	C07K 14/22; C12N 15/31		Ì
According to	International Patent Classification (IPC) or to both	national classification and IPC	
B.	FIELDS SEARCHED		
Minimum docu Int Cl <sup>6</sup> :	mentation searched (classification system followed by cla C07K 14/22; C12N 15/31	assification symbols)	
Documentation As below	a searched other than minimum documentation to the exte	ent that such documents are included in t	he fields searched
Electronic data	a base consulted during the international search (name of		terms used)
CA WPAT Medline	) Neisseria meningitidis adhesins G	REMBL ) ENPEPT ) Applicate WISS PROT PIR )	nt's sequences
C.	DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where app	ropriate, of the relevant passages	Relevant to claim No.
A	VIRGI, M. Adv. in Exp. Med and Biol. 1996. 40	8: 113-122	ALL
A	RUDEL, T. et al. Nature 1995. 373: 357-359		ALL
A	VIRGI, M. et al. Mol Microbiol. 1992. 6(19): 27	85-2795	ALL
	Further documents are listed in the continuation of Box C	See patent family ar	nnex
Spec	ial categories of cited documents:	later document published after the in	nternational filing date or
"A" docu	ment defining the general state of the art which is considered to be of particular relevance	priority date and not in conflict with understand the principle or theory w	the application but cited to nderlying the invention
"E" carli	er application or patent but published on or after "X nternational filing date	document of particular relevance; the be considered novel or cannot be con-	e claimed invention cannot nsidered to involve an
"L" docu	ment which may throw doubts on priority claim(s) hich is cited to establish the publication date of	inventive step when the document is document of particular relevance; th	e claimed invention cannot
anot	ther citation or other special reason (as specified) ment referring to an oral disclosure, use,	be considered to involve an inventive combined with one or more other su combination being obvious to a pers	ich documents, such
"P" docu	bition or other means ment published prior to the international filing "& but later than the priority date claimed		nt family
	ctual completion of the international search	Date of mailing of the international sea	rch report
7 January 19		2 1 JAN 1999	
Name and m	ailing address of the ISA/AU AN PATENT OFFICE	Authorized officer	
PO BOX 200 WODEN A	)	GILLIAN ALLEN	
AUSTRALIA		Telephone No.: (02) 6283 2266	

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 98/01031

Box 1	0	bservations w	here certain	ı claims were	found unse	archable (	Continuat	ion of item	1 of first sh	eet)	
This i		al search report	has not be	en established	in respect o	f certain cla	aims under	Article 17(2	(a) for the	following	
1.		Claims Nos.:				•. •			,		
	ш	because they r	elate to sub	ject matter not	required to	be searche	d by this A	uthority, na	mely:	• • •	
						i i jeri	•	2.35.4			
			•			1,		THE VI			
			:			•	•	•			
2.	ভো	Claims Nos.:	(A) 2, 3.	5, 6, 7, 9; (B	) 20(1) and	21					
-	X	because they r	elate to par	s of the intern	ational app	ication that	do not con	mply with the specifically	ne prescribed :	requiremen	nts to
(A)	Claims against	2, 3, 5, 6, 7, 9 themselves or	are not cle their pare	ear. They are nt organism (	essentiall Neisseria	y to polyp meningitio	eptides w dis). This	hich have i concept is	mmunologi virtually m	cal activity eaningless	y s.
							•.	com	inued		
١,		Claims Nos.:			-			COM			7
3. *?	لــا	because they	are depende	nt claims and	are not draf	ted in accor	rdance with	the second	and third se	ntences of I	Rule
Box	II (	bservations w	here unity	of invention i	s lacking (	Continuation	on of item	2 of first st	eet)		
This	Internation	nal Searching A	uthority fo	md multiple ir	ventions in	this interna	ational app	lication, as	follows:		
		•									
										:	
1		•		•			٠.				
	,	•				. 4					
1.		As all require searchable ch		l search fees w	ere timely	oaid by the	applicant,	this internat	ional search	report cove	rs all
2.		As all searchs payment of a	able claims ny addition	could be searc al fee.	hed withou	effort justi	ifying an a	iditional fee	, this Author	ity did not	invite
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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 98/01031

Box BOX 1 (2)

Antigens do not display immunological activity against themselves, or the organism from which they derive. However, as far as I can determine, these claims are intended to encompass either:

- (i) antigenic polypeptides or their encoding nucleic acids according to claims 1, 4 or 7, which provide protective immunity to an animal or human against Neisseria meningitidis infection, or
- (ii) antibodies to such antigenic polypeptides.

Since these concepts are covered by other claims the lack of search on these claims does not affect the search coverage of the claims in toto.

(B) Claims 20(1) and 21 are to any antibodies against Neisseria meningitidis. They lack support from the description as they are not limited to antibodies to the polypeptides of the invention.